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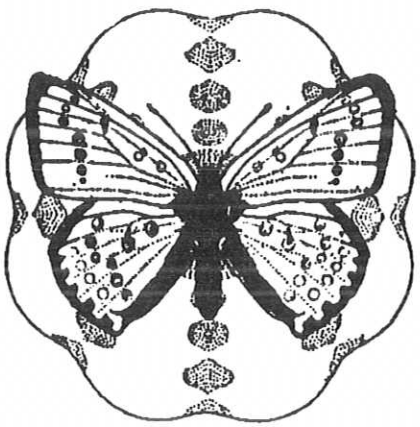

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Utrecht, 14 December 1990

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PREFACE: THE NATURAL HISTORY OF EXPERIMENTAL AND APPLIED ENTOMOLOGY IN THE NETHERLANDS

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In the Netherlands the development of experimental and applied entomology took a natural course. In the 13th century Jacob van Maerlant published the descriptive details of usefull, noxious and exotic 'worms'. By the time the Dutch Golden Age was reached internal and external morphology of arthropods had matured. The Netherlands Entomological Society was erected on October 12, 1845. It was a meeting point of amateur-entomologists among the more wealthy citizens. Applied aspects of the observations were stressed to improve the standing of the discipline at large.

Around 1900 professional entomologists, educated at the universities, appeared. But they were mainly interested in systematics and morphology. The more independant and experimentally inclined investigators were scorned at, and not until 1941 did they reach autonomy in the Netherlands Entomological Society. The period of the unlimited possibilites of biocidal intervention is then followed by concern about resistance and toxicological side effects in man, pets, and nature at large. More 'biological' methods are proposed, but in the course of the 80th research results do not reach anymore the Dutch entomological community of students and teachers of different institutions. The Section of Experimental and Applied Entomology of the Netherlands Entomological Society rejuvenated and in 1989 started to organize yearly a meeting for Dutch experimentally and/or applied working entomologists. The book under hand contains the papers presented during the Second Meeting held on 14 December 1990.

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POLLEN DIGESTION AND ITS RELATION TO THE EVOLUTION OF SOCIALITY IN THE BEES

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Keywords:
Apoidea, protein metabolism, evolution, sociality.

Summary

In this paper it is argued that pollen grains from different plants are very different in their food value for bees. This necessarily leads to implications for the rate of egg production and the development of the larvae. A number of bee species escaped from this source of variation by becoming oligolectic. Other species, possibly in order to decrease the amplitude of variation, mix pollen from different plants when preparing food for a larva.

Since the major constraint for the reproduction of bees is in brood care (nest construction and defence) and foraging, and not in the capacity to produce eggs, most bees use the opportunity to make comparatively large eggs. This is interpreted as an adaptation for shortening the development of the larva and for helping it to overcome digestive problems related to the amount of waste in the food and the closed intestinal tract.

While in solitary bees the digestive capacity for pollen is no limiting factor for reproduction, in primitively social forms this capacity soon determines the number of eggs produced by the queen. Various steps in the evolution of sociality are linked to the transfer of proteins between the adults. These steps are outlined in this paper.

INTRODUCTION

The evolution of the bees is closely linked to the evolution of the angiosperms. Angiosperms developed from the beginning of cretaceous times, and the oldest bees are from the same period. Even complex sociality occurred that early: in american amber deposits, some 80 million years old, well preserved workers of stingless bees were found (Michener & Grimaldi, 1988).

Angiosperms shifted from pollination mechanisms depending on transport by wind or water to transport by insects (and by a few vertebrates). Probably the oldest group of insects involved in pollination are the beetles, that came to the flower to eat from the relatively soft parts. Several groups of plants, like *Magnolia*, offer oil, produced in special glands in the petals, to attract such pollinators. Flies, butterflies and moths also were lured for pollination; for them nectar producing glands evolved. Like the beetles, these insects visit the flower for their own nourishment. The specialty of the bees lies in the fact that they retrieve from the flowers also the food for their larvae; they hardly become satiated, and continue to visit the flowers.

The story of the bees and the flowers is told to us at an early age, and in this story it was omitted that this relationship is far from harmonious. A given plant produces its pollen in order to pollinate the flower of a conspecific plant. Visiting insects are not interested in the pollination as such. Therefore they need to be manipulated by the plant in order to promote direct pollen transfer between conspecifics. Especially when in a

given habitat a plant species is not abundantly present, random flight of the insect would yield only a low level of pollination. This manipulation of the insect takes two forms: one is the development of specific characters in the flowers, such as distinctiveness in colouration, shape and odour, allowing the insect to discriminate this plant species from all the others it might encounter, and the other is to offer such a high reward that the insect will associate the plant species with the reward, and will specifically look after that flower type. In its choice the insect has to compare the relative yield of each plant species in order to make its food collection maximally efficient. Not only the amount available per flower, but also the costs of its flight and its collecting determines its decision. This is beautifully exemplified by Heinrich's studies on bumblebee foraging (Heinrich, 1979).

Primitive flowers are open; they consist of a large number of petals surrounding the reproductive parts of the flower. There are many axes of symmetry, and every visitor is able to get at its food. This means that all insect visitors are competitors, and that their reward generally will be low. Small insects may find it rewarding; for most others a visit to such flowers will soon become uneconomic. This induces various evolutionary lines in plants. One is that many flowers are offered in close vicinity (Umbellifera, for instance), stimulating an insect to visit them in a rapid succession. Another is the development of protective devices, so that the nectar can accumulate, until a specific pollinator opens the flower (Labiatae, Orchidea) by its specific dimensions or weight and finds an extraordinary reward. A long and slender corolla or specially designed tubular flower structures have the same function. Such developments incite evolutionary changes in the group of pollinators as well. This process of coevolution is the subject of many fine books and papers. I may refer here to Baker & Hurd (1968), van der Pijl & Dodson (1966) and Barth (1985). As a result of this complex competition we have small and large bees, generalists and specialists in their flower visits, altogether over 20.000 species (Michener, 1974).

POLLEN

Bees obtain all their food from flowers; nectar to provide the energy, and pollen to provide the proteins. Some plants offer oil instead of nectar and some bees use this oil instead of sugar, even to feed their larvae (Vogel, 1974). While nectar and oil are secreted to lure the pollinator, and selection processes are influenced only by this single function, pollen is produced primarily for pollination. The properties of the pollen and the way it is produced is the joint product of selective pressures related to this pollination and its collection and use by the pollinator. As a consequence, pollen is probably a much more variable kind of food than nectar or oil.

This variation concerns various characteristics. In the first place there is tremendous variation in shape and size of the pollen grains of different plants, leading to differences in volume of 6 orders of magnitude: *Myosotis* has pollen grains of 0.065×10^{-6} mm^3, *Aesculus* of 5×10^{-6} mm^3 and *Cucurbita* of 5.000×10^{-6} mm^3. This is important because the wall of the pollen grain is indigestible, only the living protoplast can be used by the bee. Differences in volume then relate to differences in the ratio protoplast : waste, an important factor in the digestive efficiency of the intestine.

Since the pollen wall is so resistant, digestion probably takes place through the germinating pores of the pollen grain. These are small openings, where the protoplast is protected only by a relatively thin membrane. Enzymes may attack this membrane and enter the pollen grain through diffusion. After the degradation of the cell structures the products of digestion, again by diffusion, have to leave the casket through the narrow pores. Dimensions of the pollen grain have a strong impact on the rate of this process.

Another source of variation lies in the composition of the protoplast. There is variation with respect to amino acid composition of the protein. For instance, in *Taraxacum*, there is no arginine, an essential amino acid for the honeybee (Herbert et al., 19xx). Others, like *Zea mays*, contain large amounts of starch granules, at the expense of

protein. *Pinus*, a wind pollinated plant, has pollen grains with air sacs. All this lead us to conclude that even size is no good reference for possible yield. I may refer to Stanley & Linskens (1974) for further sources of variation.

For bee larvae the consequences of this high variability are even more important than for adults, because bee larvae are characterized by having a closed intestine. It is only in the last instar larva that the connection between the ventriculus (midgut) and the rectum is made, and as a consequence the larva has to carry all the waste material in its ventriculus.

Given the impact of variation in pollen on the efficiency of pollen digestion, we understand the two strategies developed within the bees. One is that a species stabilizes its food quality by becoming a specialist in its foraging. Such oligolectic bees collect their pollen from a very limited number of plants. They have to synchronize their life cycle with the blossoming period of their food plants. In Westrich's (1989) beautiful book several of such species can be found.The alternative is to mix various pollen types, in order to reduce the fluctuations in yield that would otherwise occur.

PROTEIN METABOLISM

Once the protein is digested, it penetrates the midgut wall and enters the haemolymph. From there it reaches the fat body, a diffuse type of tissue covering many parts of the integument. Fat body cells are storage cells, and at the same time these cells are capable to transform one protein into another. The activity and functioning of the fat body at a given time is probably controlled by hormonal systems. In the larvae the fat body accumulates the building material for the adult, the cells becoming active in the pupal stage. In the female adults they produce the specific proteins (vitellogenins) that migrate, through the haemolymph, towards the ovaries where these proteins are incorporated in the growing oocytes (Engels, 1974).

OOGENESIS AND EGG LAYING CAPACITY

Formation of eggs, once the meiosis took place, is a complex process. The meiotic division leads to the formation of an oocyte and 48 trophocyte cells. The latter develop from the polar bodies through 4 subsequent cell divisions. Together oocyte and trophocytes form a unit, surrounded by a layer of follicle cells. They grow by accumulating yolk material through their cell wall. In the meantime they descend the ovariole. Then the trophocyte cells fuse with the oocyte, causing a rapid increase in size of the latter. The follicle cells now secrete the hard follicular envelope, separating the ooplasm from the environment, and the egg is ready to be laid. Supposedly, a certain minimum period of time is necessary for the completion of the egg; the reproductive capacity of a female bee is then further determined by the following factors: the digestive capacity, determining the flow of proteins towards the fat body; the capacity of the fat body to synthesize vitellogenins; the carrying capacity of the transport system and the number of ovarioles in the ovary. Most bee genera have 3 or 4 ovarioles.

However, the full capacity of this physiological machinery is probably seldomly used. This is because bees construct elaborate nests, which they defend against competing conspecifics, predators and parasites, by staying at the nest entrance, leaving only occasionally to collect food. Nest construction and defence are major factors in the time expenditure of a female. For every larva to be produced many flowers are to be visited, and this too is very much time consuming. In most bees the food in a cell is transformed into a bee bread, the mixture of pollen and nectar, and only after this is completed the female will produce an egg. In most solitary bee species a female produces only a very limited number of eggs in her life time, somewhere between 6-15. Probably related to this is the fact that many bee species produce extremely large eggs (Iwata, 1964;

Iwata & Sakagami, 1966). The ratio between the length of the egg compared to that of the bee even obtains values of 0.5 in the carpenter bees.

So far no specific function has been attributed to this large egg. It is my suggestion that this function might be twofold. The first is, that it reduces the time needed for development. The extra amount of protein is already available in the yolk, and the larva doesn't need to digest the equivalent amount of pollen. The second reason might be that the volume of waste material resulting from pollen digestion, and the closed intestine, is a constraint on its own, especially in minute larvae.

SOCIAL EVOLUTION

In several taxonomic groups of the Apoidea an onset to sociality is present. In most of these cases this only led to a primitive form of sociality, consisting of a temporary or permanent cohabitation of a few females in a nest. In two groups, the Halictidae and the Apidae (to which belong the honeybees, the stingless bees and the bumblebees) more complex colonies and, in the latter, even permanent sociality arose. In the literature several selective pressures have been indicated to promote such an evolution. These are the scarcity of suitable nesting sites, leading to aggregated nesting, the value of an already existing nest enabling its re-use, the necessity to defend a nest against competitors, and also the impact of kin selection, the indirect way of transmitting an individuals' genes to future generations by assisting a close relative in its breeding efforts. I like to add the impact of limiting physiological factors with respect to protein acquisition.

As soon as a division of labour develops in a community of females, implying one female producing most of the offspring of a nest and the others operating as helpers, the reproducing female has to increase the number of eggs laid in comparison to a solitary species. The digestive capacity of the intestine soon will become the limiting factor. As a first step to increase the delivery rate for protein I consider the fact that in these initial levels of sociality several females produce eggs, but through competitive interactions the eggs of subordinate females are eaten by the dominant female. Such eggs are no doubt a better protein source than the original pollen. This means that the digestive capacity of the subordinated ones serves to augment the rate of egg laying in the dominant.

This interpretation is in agreement with behavioural data that show that a dominant female does not attempt to prevent egg laying by others, but that she intervenes at a later stage. Indeed, if the dominant profits from the eggs of the subordinates, she should stimulate rather than inhibit them to lay eggs. To interprete the absence of inhibition as demonstrating the incompleteness of dominance, as can be found in the literature so often, is unjustified.

Egg eating occurs in several groups, from the socially primitive associations of carpenter bees (Sakagami & Maeta, 1987; Stark et al., 1990), several social Halictidae (Kukuk, in manuscr.) up to the highly social stingless bees. In the latter group a beautiful evolution of worker egg laying encompasses all possible stages. In normal *Scaptotrigona* colonies workers are involved in the laying of fertile eggs, from which males develop (Beig, 1972). In *Melipona* workers generally produce semi-fertile eggs, positioned on the food in the same way as real eggs, and which are always eaten by the queen (Sommeijer et al, 1981); if the colony has lost its queen these eggs are fully developed and produce males. In other species the trophic eggs are laid at the cell rim instead of onto the food; and in *Plebeia* workers 'offer' an egg to the queen when they suddenly encounter her on the comb (van Benthem, 1987). In still other species worker ovaries remain unactive, even in the absence of the queen.

In the primitive eusocial bumblebees egg eating does not play such a role. The colony develops through the rearing of three distinct broods (Duchateau & Velthuis, 1988). The first brood is reared by the still solitary queen. This brood emerges and helps the queen in the rearing of the second brood. The third brood is generally by far the largest one and is produced in a continuous series of egg cups. At this time the queen appears to be at her maximum egg laying rate; she does not obtain any help from the

workers in producing these eggs. Workers activate their ovaries, and within a week after emergence their ovaries may contain ripe eggs. However, these eggs are not laid, but resorbed again for a period of about three weeks (Duchateau & Velthuis, 1989). Then the dominance of the queen weakens and several workers at the same time start laying eggs. Intense competition involving egg eating ensues and prevents a further increase of the population. While the first and second brood consists exclusively of workers, the third brood also includes males and young queens. Worker laid eggs, if they survive, develop into males, but their number seems to be very limited. As a consequence of these mechanisms a bumblebee colony necessarily contains never more than a few hundred individuals, and often the maximum number remains below a hundred.

Colonies of several stingless bee species and of the European honeybee, *Apis mellifera*, however, may contain 10,000 till over 50.000 individuals, and all or almost all are direct descendants of the single queen. How does the queen manage to build up and maintain such a large population? Several adaptations can be mentioned.

The first adaptation concerns the increased capacity of the ovary by anatomical modifications. The stingless bees retained the 4 ovarioles per ovary, characteristic of many bees. However, there occurred an enormous elongation of the ovarioles; in a laying *Melipona rufiventris* queen the ovarioles were at least 8 times the length of the abdomen. This allows a more rapid movement of the oocytes through the ovariole, and consequently leads to a larger output. A second adaptation lies in the longevity of the worker. On average, this may be 6-12 weeks, about twice as long as an average worker of *Apis mellifera*. Replacement rate needs to be only half as high.

In the honeybees queens have a very high number of ovarioles: in *A. florea* and *A. cerana indica* this is about 70 per ovary; in *A. dorsata* it is 130, and in *A. mellifera* even 180 (Velthuis, 1976). The ovarioles are relatively short, only about the length of the abdomen. In addition, eggs are rather small.

While stingless bee queens often receive help from the workers in that the workers provide trophic eggs, in the honeybees a new development leads to a comparable transfer. This concerns the hypopharyngeal gland of the worker. This gland produces a secretion rich in protein, and this secretion is fed to the tiny larvae in worker and drone cells during the first 3 days of their development; queen larvae even receive this high quality nourishment during their entire larval period. The same secretion is used to feed the queen. The hypopharyngeal gland is present also in stingless bees, bumblebees and even in various solitary bee species. In various stingless bee species, the amount of free protein and amino acids in the liquid fraction of the provisions in a brood cell is rather low (Hartfelder & Engels, 1989). In these cases the hypopharyngeal gland probably serves as a source of digestive enzymes; in the course of evolution such enzymes may then be secreted and be mixed with the food for the larvae. In the honeybees the enzymatic properties become less important than the proteinaceous character of enzymes. However, foraging workers of *Apis* still use the gland for the production of an enzyme, capable of converting disacharides into monosacharides.

This functional modification of the hypopharyngeal gland probably is a very important one for other aspects as well. It brings about a more stabilized food for the larvae, and development of the individuals can be more precisely regulated. It also enlarges the scope of plants that can be visited by the foragers of the colony. Even if the quality of a given pollen is poor, the digestion of it in the worker and the subsequent transformation in a secretion means that nevertheless a stabilized food is produced. This may explain why honeybees can be observed to collect even the spores of fungi and the dust of charcoal, a behaviour not known from other bees.

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PREDATORY MITES (ACARI; GAMASIDA) AS SPECIFIC DUNG INDICATORS IN ARCHAEOLOGY

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Keywords:
Acaro-archaeology, Gamasida, excrements, dung indication.

SUMMARY

The preliminary results of a study on characteristic predatory mite faunas in the dung of domestic animals form the basis of a two step identification method for archaeological dung deposits.

INTRODUCTION

The study of the remains of mites (Acari) found in an archaeological context provides valuable ecological information (Schelvis 1990a). So far this acaro-archaeology has mainly focussed on the interpretation of finds of moss mites (Oribatida). The remains of representatives of this relatively well studied order of mites have been shown to be highly suitable as a basis for palaeo-environmental reconstructions (Schelvis 1990b). There are, however, more archaeological questions to be answered with the aid of acaro-archaeology. For instance: Does this particular archaeological deposit contain animal dung? and if so: Which of the domestic animals produced this dung?

Predatory mites forming the order Gamasida generally prefer habitats rich in decaying organic matter such as compost heaps, cess pits and dung hills. Furthermore, many of these predatory mites have more or less specialised feeding habits. Differences between invertebrate faunas living in various types of animal dung will be reflected in characteristic predatory mite faunas. It is obvious that the archaeological questions concerned with animal dung could be answered through the study of the remains of these predatory mites. Unfortunately, most acarologists have mainly studied predatory mites in 'natural' habitats. When they do record a member of the Gamasina in animal dung they usually refer to the habitat simply as dung or excrements without stating which animal produced this dung. Therefore, to obtain these data it was necessary to perform a detailed study on recent 'dung mites'. Supplemented with the scarce literature on predatory mites in dung this study provides the basis for the identification of archaeological dung deposits. Although the study has not yet been completed the preliminary results, presented in this article, already illustrate the usefulness of predatory mites in acaro-archaeology.

MATERIAL AND METHODS

Samples are taken from the excrements of five domestic animals: cattle, sheep, horse, pig and chicken. These samples are taken indoors in stables and sheds as well as outdoors from dung hills and in 'field situations'. Care is taken to sample excrements which vary in age. Usually nine subsamples are taken: three from fresh droppings, three from somewhat older droppings and three from old, mouldy droppings. The predatory mites contained in these subsamples are extracted by means of a Berlese Tullgren funnel. The predatory mites of the cohort Gamasina are identified with the aid of Karg (1971) and the representatives of the cohort Uropodina are identified with the aid of Hirschmann & Zirngiebl-Nicol (1960-1967) and Karg (1989). Species which can not be identified in this way are given a type number and descriptions are being made. These descriptions, which are not given in this article, together with the permanent slides made of these type specimens can help to identify archaeological remains of predatory mites.

On the basis of the identifications two lists of species are being made comprising those predatory mites which are thought to be characteristic for dung. One list containing those mites which were found in the dung of at least three different animals but never in any of the 12 non-dung samples. These predatory mites are referred to as General Dung Indicating (GDI-) species (Fig.1). The other list of dung indicating species is made up of those predatory mites which were found in the dung of two different domestic animals and again never in any of the non-dung samples. These species are referred to as Possible Dung Indicating (PDI-) species. This latter list is supplemented, on the basis of literature references, with those species which are known to occur commonly in a variety of animal excrements.

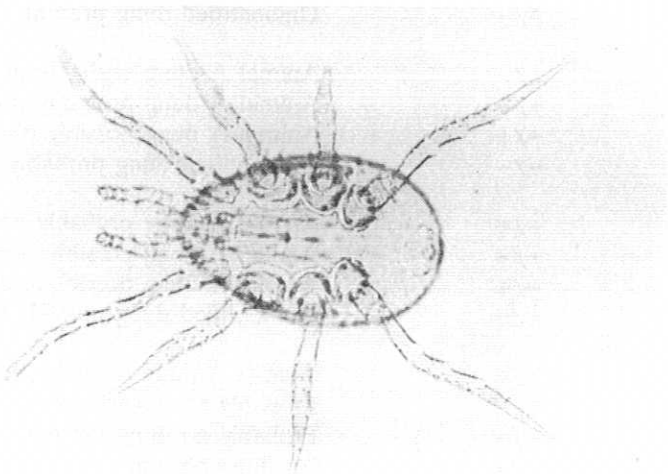

Fig. 1 *A typical dung indicating predatory mite,* Crassicheles holsaticus.

The next step in the interpretation of an archaeological dung sample is to try and identify the producer of the dung. To do so lists are being made of those predatory mites which are thought to be characteristic for the dung of a particular domestic animal. A predatory mite is considered to be a Producer Indicating (PI-) species for animal A when it is found exclusively in the dung of animal A. The lists of Possible Producer Indicating (PPI-) species are made up of those mites which were found not only in the dung of one particular animal but also in the excrements of other animals. These predatory mites are considered to be PPI-species of producer A only when their relative abundance is more than three times higher in the dung of animal A than in the dung of any other animal. These latter lists are again supplemented, on the basis of data taken from literature, with those predatory mites which are referred to as occurring only in the excrements of a particular domestic animal.

The interpretation of an archaeological dung sample is now a simple two step procedure. First it is checked if there are any dung indicating species present. Subsequently the presence of producer indicating species for animal A is recorded. Table 1 illustrates this method and gives the eight possible conclusions drawn from the various results.

Step 1 Dung Indication GDI/PDI-species	Step 2 Producer Indication PI/PPI-species	Conclusion
+/+	+/+	Animal A dung present
+/+	+/-	Animal A dung probably present
+/+	-/+	Animal A dung possibly present
+/+	-/-	Unidentified dung present
+/-	+/+	Animal A dung probably present
+/-	+/-	Animal A dung probably present
+/-	-/+	Animal A dung possibly present
+/-	-/-	Unidentified dung probably present
-/+	+/+	Animal A dung probably present
-/+	+/-	Animal A dung possibly present
-/+	-/+	Animal A dung possibly present
-/+	-/-	Unidentified dung possibly present
-/-	+/+	Animal A dung possibly present
-/-	+/-	Probably no dung present
-/-	-/+	Probably no dung present
-/-	-/-	No dung present

Table 1 *The eight possible conclusions drawn from the presence or absence of dung indicating species and producer indicating species for animal A in an archaeological dung sample. (+ = present, - = absent)*

RESULTS

After the identification of more than 7000 predatory mites from eleven recent dung samples the following two lists of Dung Indicating and Possible Dung Indicating species (Table 2) were made on the basis of the criteria given in the Material and Methods section. Species which were supplemented on the basis of literature data are marked *.

General Dung Indicating species	Possible Dung Indicating species
Uropoda orbicularis	*Trichouropoda orbicularis* *
Crassicheles holsaticus	*Uroobovella marginata*
Macrocheles glaber	*Macrocheles vagabundus*
Halolaelaps subtilis	*Trachygamasus gracilis*
Parasitus lunaris	*Ameroseius plumosus* *
Parasitus coleoptratorum	*Halolaelaps porulus*
Parasitus fimetorum	*Halolaelaps type R19b*
Parasitus type R18a	*Dendrolaelaps punctum*
	Dendrolaelaps arvicolus
	Parasitus mustelarum
	Parasitus mycophilus
	Parasitus numerus

Table 2 *General and Possible Dung Indicating Species.*

On the basis of the same eleven samples the following ten lists of Producer Indicating and Possible Producer Indicating species (Table 3) were made for the specific identification of the dung of five domestic animals. Species supplemented on the basis of literature data are again marked *.

Cattle Indicating species	Possible Cattle Indicating Species
Uroobovella crenelata	*Uropoda orbicularis*
Macrocheles vernalis	*Macrocheles peniculatus* *
Macrocheles pavloskii	*Macrocheles decoloratus* *
Halolaelaps punctulatus	*Pachylaelaps sculptus* *
Parasitus talparum	*Ameroseius corbiculus* *
	Halolaelaps communis *
	Halolaelaps tuerkorum *
	Halolaelaps saproincisus *
	Dendrolaelaps willmanni *

Sheep Indicating Species	Possible Sheep Indicating Species
Uroobovella fimicola	*Parasitus numerus*
Pachylaelaps siculus	*Parasitus mycophilus*
Dendrolaelaps strenzkei	
Parasitus mammillatus	
Parasitus hyalinus	

Horse Indicating Species	Possible Horse Indicating Species
Uroobovella varians	*Uroobovella marginata*
Uroobovella difoveolata	*Amblyseius fraterculus* *
Nenteria floralis	*Ameroseius insignis* *
Nenteria stammeri	
Macrocheles insignitus	
Dendrolaelaps stammeri	
Pergamasus vagabundus	
Parasitus eta	
Pig Indicating species	**Possible Pig Indicating Species**
Macrocheles merdarius	*Parasitus mustelarum*
Gamasodes bispinosus	*Parasitus lunaris*
Parasitus type R20a	
Poultry Indicating Species	**Possible Poultry Indicating Species**
Discourella cordieri	*Macrocheles muscaedomesticae*
Nenteria breviunguiculata	*Halolaelaps subtilis*
Trichouropoda ovalis	*Halolaelaps quadricavatus* *
Trichouropoda longiovalis	
Trichouropoda type R19a	
Macrocheles matrius	
Androlaelaps casalis	
Holostaspis heterosetosa	
Amblyseius obtusus	

Table 3 *Producer and Possible Producer Indicating Species for the excrements of cattle, sheep, horse, pig and poultry.*

The results of the acaro-archaeological study of a 14th century sample taken in 1987 at the Martinikerhof in Groningen (the Netherlands) will serve as an example of the interpretation of an an archaeological dung sample on the basis of the remains of predatory mites. This sample contained the remains of 178 mites including 61 predatory mites and 103 moss mites (Schelvis 1989). The identification of 42 of these 61 predatory mites (= 69%) resulted in the following species list:

Uroobovella pyriformis	15
Nenteria stammeri	12
Uroobovella difoveolata	12
Uroobovella marginata	2
Ameroseius plumosus	1

There are two Possible Dung Indicating species present (*U. marginata & A. plumosus*), two Horse Indicating species (*N. stammeri & U. difoveolata*) and one Possible Horse Indicating Species (*U. marginata*). According to table 1 the conclusion is that there is probably horse dung present in this sample.

DISCUSSION

It should be stressed that the above presented results are preliminary ones. Dung samples from some domestic animals has been studied more thoroughly so far than the excrements from others. Therefore, the species composition of the (Possible) Producer Indicating lists could be different when all the samples have been taken and studied. This could also result in the addition of new General or Possible Dung Indicating species.

Archaeological samples never consist entirely of animal dung, they are always mixed to a varying degree with sand, peat, clay and/or decomposing botanical matter. This results in the introduction of remains of mites which are not characteristic of dung. Since the amount of mixing is not known it is not possible to characterise an archaeological dung deposit on the basis of the relative abundances of General or Possible Dung Indicating species. Therefore, the presence or absence of these species was chosen as the criterion for the presence of animal dung in archaeological samples.

It is, ofcourse, possible that the excrements of different animals become mixed within one deposit. In the sample from the Martinikerhof in Groningen only (possible) horse indicating species were found. How would the interpretation of the results be if other Producer Indicating species would also have been found in this sample ? In that case the relative abundances of the species found may help in the interpretation. Low relative abundances of Possible Producer Indicating Species characteristic for animals other than the horse will not alter the general conclusion. On the other hand, when there are Producer Indicating Species for animals other than the horse present with a considerable relative abundance the conclusion will have to be that there is dung from more than one animal species present in the sample.

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LICE AND NITS (*PEDICULUS HUMANUS*) FROM MEDIEVAL COMBS EXCAVATED IN THE NETHERLANDS

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Keywords:
Entomo-archaeology, hair combs, middle ages, pediculosis.

Sucking lice forming the insect order Anoplura parasitize many species of birds and mammals. Man is affected by the head louse (*Pediculus humanus*) and the crab louse (*Pthirus pubis*). Apart from the irritation caused by these parasites they are potentially dangerous as vectors of disease, such as typhus fever. Man has always tried to minimise these infestations, originally by means of manual removal and later with the aid of specially designed delousing implements such as nitcombs. It is historically documented that during the middle ages pediculosis was a common phenomenon among all classes of society. From the Netherlands any proove of this in the form of archaeozoological records of lice was lacking. Recent research, however, on medieval combs has resulted in the first archaeological finds (Fig.1) of the head louse (*Pediculus humanus*) in the Netherlands (Schelvis in press).

Archaeological finds of lice and nits in combs are very rare. Kenward (1985) reports on nits, found on a Viking age comb from York, most likely belonging to the genus *Damalinia*. An other publication concerning archaeological finds of lice and nits between the teeth of combs is by Mumcuoglu & Zias (1988). They found the remains of head lice and their eggs on 12 out of 24 combs made of boxwood. In this case the arid climate of the Middle Eastern desert resulted in the good state of preservation of the organic remains. Under more humid conditions, such as normally found in the Netherlands, the state of preservation of the organic remains was found to be equally good. So far I have found the remains of head lice and nits on 7 out of 19 Dutch medieval combs.

The remains of head lice have been found on combs made of boxwood, bone, antler and horn. Nits, however, have so far only been found on a comb made of boxwood (identification W.A.Casparie, B.A.I. Groningen). This 15th century comb contained the remains of 125 nits and 2 lice. Although combs made of other materials were, with regards to the mean Distance Between Teeth (DBT), sometimes nearly identical to this particular comb they never contained any remains of nits. The fibrous surface of the boxwood is apparently a good substrate for 'catching' nits. Still, the mean DBT does appear to be the main factor determining the presence or absence of the remains of lice. All lice remains have so far been found in combs with a DBT of less than 0.7 mm.

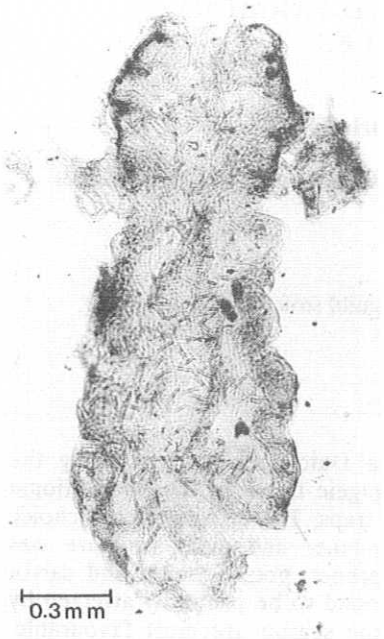

*Fig.1 Archaeological remains of human headlouse (*Pediculus humanus*) from the 17th century.*

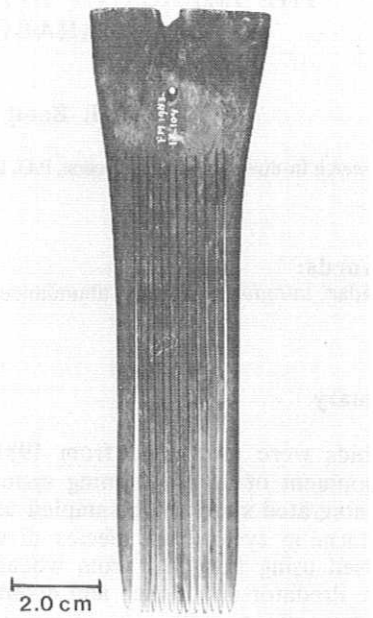

*Fig.2 A 'wool'comb which was found to contain the remains of human headlouse (*Pediculus humanus*).*

The main application of the study of lice remains in combs is not the recording of pediculosis in the past. The most useful information derived from this research has an archaeological character. It can be used to designate the use of certain types of combs. There is, for instance, a type of long comb (Fig.2) which is found in northwestern Europe made out of metapodials of cattle. These combs, which were produced from the 9th till the 15th century, are always refered to in archaeological literature as carding combs or wool combs. Since I have found the remains of *P.humanus* on 4 out of 11 of these combs it is obvious that this interpretation is not correct. They are simply morphologically different hair combs.

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THE IMPACT OF INTEGRATED FARMING ON CARABID BEETLES

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Keywords:
Carabidae, integrated farming, abundance, diversity, guild structure, agro-ecology.

Summary

Carabids were monitored from 1981-1987 in a Dutch project concerning the development of arable farming systems. The epigeic fauna of the conventional and integrated system was sampled using pitfall traps. The effects of crop choice and farming system on species diversity, abundance and guild structure was analysed using trap-data from wheat, pea, sugarbeet, potato, onion and carrot fields. Predator abundance and diversity were found to be primarily affected by crop type. Crops with a good cover early in the season are most favourable. Within crops abundance and diversity of carabids is enhanced by integrated farm management.

INTRODUCTION

At present agriculture is under great pressure to reduce the input of chemical fertilizers and pesticides in order to diminish detrimental effects on the environment.

The necessary transition of conventional to integrated systems does not only mean a reduction of chemical inputs but also major changes in cropping systems and crop management practices. Since one of the intentions of integrated farming is to increase the ecological resilience of the agroecosystem against pests and diseases, it is worthwhile to study the impact of these changes on natural enemies (Edwards & Stinner, 1990).

In agroecosystems carabid beetles are probably the most important and best studied group of natural enemies and their role in natural control of insect pests has been well documented (Thiele, 1977; Luff, 1983, 1987). Although the importance of insect predators in sustainable agriculture is widely accepted, the way farming systems affect carabids is poorly understood.

Recently, the carabid fauna of conventional and biological farming systems has been compared (Dritschilo & Erwin, 1982; Holopainen, 1983; Hokkanen & Holopainen, 1986; Kromp, 1989; Booij & Noorlander in press). The concept of integrated farming, however, is less different from conventional farming than that of biological or organic farming and its impact on carabids probably is more subtle. In this paper the effect of integrated farming on carabid abundance and diversity is analysed and the role of different agroecological factors is discussed.

MATERIALS AND METHODS

The carabid project was part of the Dutch research project "Development of Farming Systems" at the experimental farm at Nagele (Vereijken, 1989). The experimental farm includes a conventional, and an integrated farming system of about 20 hectares each, with individual fields of on average 3 hectares. The conventional and integrated systems are arable farms with an identical 4 year crop rotation for the main crops. In the integrated system, the input of fertilizers and pesticides is reduced by about 50%. In the integrated system, pesticide applications are based on damage thresholds and broad spectrum pesticides are avoided. Weed control in the integrated system is less intensive.

In each of the systems ground dwelling predators were sampled in winterwheat, potato, sugarbeet, pea, onion and carrot in the period 1981-1987. Sampling was done with 4 pitfall traps per field during the main growing season (May 1 - August 15). Traps were half-filled with a 4% formalin solution and emptied weekly and their contents analysed.

The response of predators to system effects or crop effects was measured in terms of abundance, species diversity and guild structure. It is assumed that there is a fair correlation between activity-density and real abundance when using continuous sampling throughout the season (Baars, 1979). As a diversity measure the average number of species per trap per season (species density) is used here. To analyse differences in quild structure, yearly totals per field were log transformed and analysed by principle component analysis using the ordination package Ordiflex.

RESULTS

In figure 1 the average abundance and species density of carabids are arranged by crop, and differences between systems are indicated within each crop.

The abundance of carabids was found to differ significantly between crops. They were most numerous in winterwheat, whereas carrot and onion seemed to be very unfavourable crops. The ordering of crops suggests that the growing pattern of the crop is of crucial importance. The presence of cover during winter and early spring as in winterwheat, seems to be very favourable whereas late and open crops like carrot and onion are unattractive for most species.

The required availability of food, shelter and a moderate microclimate is probably related to the amount and duration of crop cover. These findings are consistent with earlier reports where it was shown that winter crops like cereals are more favourable than root crops like potato and sugarbeet (Thiele, 1977).

Carabid species density was found to follow the same pattern as the abundance data (Fig. 1b). Differences between crops are more obvious than those between systems. Here also winterwheat proved the most favourable crop and onion and carrot again came out as poor crops.

Approximately 30 species were found in each farming system every year. This suggests that there is no influence of integrated farming on diversity. However, species density was consistently higher in the integrated farming system in all crops. This suggests that low-density species occurred more abundant and widespread in the integrated system.

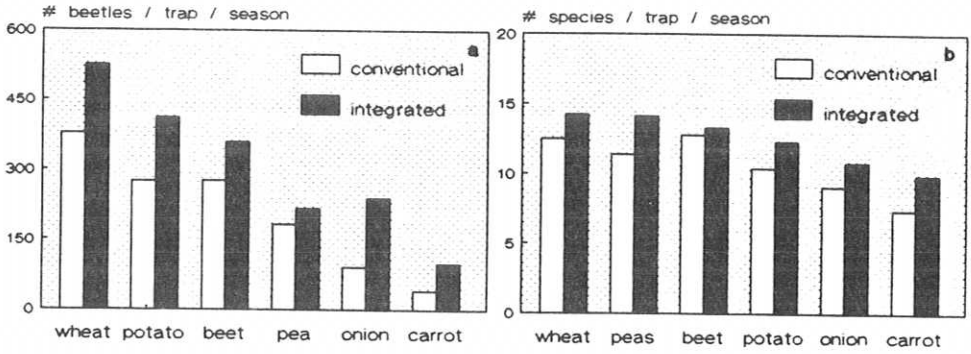

Fig 1. System and crop effects on carabid abundance (a) and diversity (b).

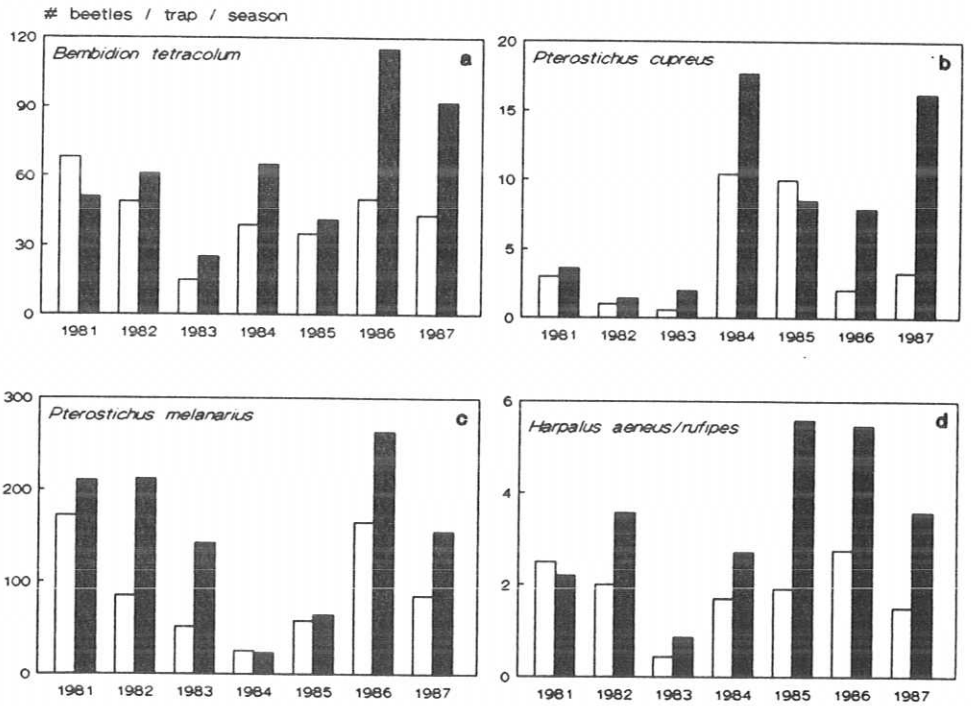

Fig 2. The response of some characteristic carabid species to different crops and farming systems. Open bars: conventional, closed bars: integrated.

Within each crop, integrated farming appears to stimulate carabid abundance. The differences found are of the same order as those found between crops and consistent for several species over the years (fig. 2). Although the differences between systems are mainly due to the abundance of the most dominant species *Pterostichus melanarius*, the trends remain the same when this species is excluded from the analysis. Both *P. melanarius* and *P. cupreus* have consistently higher numbers in the integrated system over the years. The only year that *P. cupreus* was less abundant in the integrated system was after a double application of parathion against pea weevil.

The apparent increase of seed-feeding species *Harpalus rufipes*, and *H. aeneus* (fig. 2d) by integrated farming may be due to a greater availability of weeds. Also *Bembidion tetracolum*, which is abundant in spring (fig. 2a), is probably favoured by presence of more shelter in the integrated system.

When looking at total abundance, the response of individual species to crops and systems can be greatly obscured. We certainly found species with a very obvious response pattern to crops and systems. The combined response of different species in the guilds can be easily summarized by differences in species composition. Principle component analysis appeared to be an effective tool in this respect.

The result of the analysis is shown in figure 3. The horizontal axis in figure 3a indicates the primary underlying trend. That axis corresponds very well with the horizontal clustering of crops, which is indicated in this figure. It is obvious that crops which differ most with regard to carabid abundance and species density, are also most different in their species composition. Again, there is a contrast between winterwheat and peas on the left, and onions and carrots on the right side of the figure.

The same data set of year samples is depicted again in figure 3b, but now the farming systems are indicated. The secondary (vertical) axis corresponds with a subtle separation of the systems. It seems that the greatest differ-ences are in the extremes for quite closed and very open crops. Since there are no species which occur exclusively in one of the systems, differences should be mainly ascribed to shifts in relative abundances.

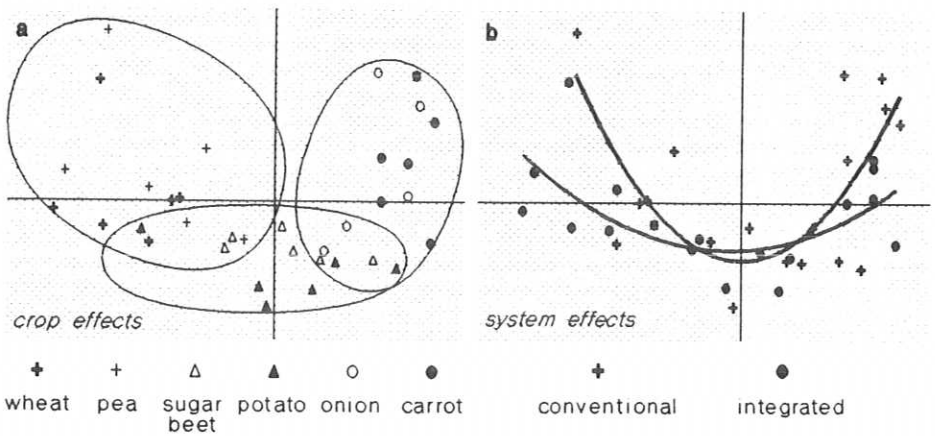

Fig 3. Principle component analysis of carabid guilds in agricultural fields. Primary effects of crop type (a) and secundary effects of farming systems (b).

DISCUSSION

The results presented in this paper show that diversity, abundance and guild structure of polyphagous predators in arable fields are primarily determined by crop type. Apparently, there are good crops like winterwheat harbouring many species at high densities and poor crops like onion with only a few species at low densities.

Since the relative abundance of different species varies between crops, crop diversity in the agricultural landscape contributes to faunal richness, but not necessarily to overall carabid density. The latter aspect is mainly determined by the percentage of favourable crops.

Predator abundance and diversity within each crop is increased by integrated farming. The impact of integrated farming may even be underestimated in this study because of the size of the experimental farm. Due to the mobility of many field inhabiting species, local population crashes or even species extinctions in the conventional system may be buffered by the adjacent integrated system. This argument also holds for the differences between crops. The close connection between carabid abundance and diversity in different crops may be explained by a general reduction in population levels in the poor crops, by which the chance of trapping low-density species becomes very low.

As a consequence of the system approach of the Nagele project care must be taken in the interpretation of the data. For example it is very difficult to unravel the effect of a reduction in pesticide use from other crop management practices (Brown & Stephenson, 1990). Although the effect of pesticide applications can be severe, it is often detectable for a few weeks, after which populations recover (Booij & Noorlander, 1988). In many instances the presence of unsprayed fields nearby may facilitate this phenomenon. Large scale applications in conventionally managed farmland can be expected to have longer lasting effects. Although the effects of single applications could often not been demonstrated, it seems likely that the total package of chemicals is responsible for part of the difference in abundance between the systems.

Apart from direct toxic effects of pesticides, indirect effects of chemical and non-chemical factors may be equally important. This includes the reduction of prey by pesticides, and the removal of weeds which are an important source of shelter and food for carabids. The presence of weeds is likely to have a positive effect on many species (Purvis & Curry, 1984; Powell et al. 1985).

Many field inhabiting predator species migrate between favourable and unfavourable habitats within the landscape. Shelterbelts, grassy field margins, unsprayed headland and natural elements in the landscape are not only important for recovery after disturbance but they are also essential for many species which need coverage for overwintering (Sotherton, 1984, 1985; Wallin, 1985). In this respect winter cover crops and winter-sown cereals may also enhance predator survival.

The main idea arising from the results presented here and the conclusions reached, is that stimulating abundance and diversity of beneficial insects in the agricultural landscape cannot be attained by only lowering chemical input in the crops grown. The agroecological infrastructure has great impact on the maintainance of abundant predator populations and its relation with carabid survival and performance should therefore be further studied.

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NATURE RESTORATION AND THE ROLE OF THE MESOFAUNA IN DECOMPOSITION OF ORGANIC MATTER

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Keywords:
Mites, springtails, decomposition process, nutrient impoverishment, feeding guilds, life history tactics.

Summary

As to nature restoration in abandoned agricultural areas on sandy soils a malfunctioning appears in the process of decomposition of organic matter. In this paper a possible explanation is presented for the resulting litter accumulation and felting of the grassland vegetation. The soil mesofauna of the areas to restore has been influenced dramatically by agricultural management, as appears from the change in fractions of microarthropod life-history tactics. As a result of this change many species which stimulate decomposition are absent in agricultural areas. These species are not phoretic, have hardly any dispersal capacity, and will thus not reach the abandoned agricultural area soon. A possible solution is presented, to be carried out after a small-scale experiment.

INTRODUCTION

Before long an increasing surface of agricultural areas will be abandoned because of too high production quota for many agricultural products in the European Community. These areas should be made suitable for the restoration of natural communities and processes. Experience has been obtained in abandoned agricultural areas in The Netherlands for some years (Cranendonk, Soerendonk). A problem during the proces of nutrient impoverishment is the felting of the grassland vegetation after some years. The removal of vegetation production is less than during the former agricultural situation, so much organic matter remains as litter. The accumulation of litter indicates a malfunctioning of the decomposition process. In this paper I give a possible explanation for the malfunctioning of decomposition in abandoned agricultural areas on sandy soils. Because of the naturally low pH, these soils have usually no high densities of earthworms, isopods and diplopods as primary decomposers among the macro-invertebrates. Primary decomposers such as bacteria and fungi are very good dispersers and their absence will

not be the cause of a lower decomposition rate. The key factor here is probably found in the mesofauna (nematods, mites and springtails); these amimals influence the activity of the primary decomposers, not just by fragmentation of the litter but also by grazing on bacteria and fungi and recycling spare elements quickly in that way (Van der Dijk & Jansen 1977, Bååth et al. 1981, Ingham et al. 1981, Hanlon 1981, Hanlon & Anderson 1979).

THE MESOFAUNA OF AGRICULTURAL AND NATURAL GRASSLANDS

Siepel & Van de Bund (1988) showed an enormous difference in the species composition of unfertilized natural grasslands and highly fertilized agricultural grasslands. The reason for this shift in species composition lies in the interaction of the grassland management and the species life-history tactic. Species having a slow juvenile development and species having asexual reproduction (thelytoky) become less abundant under the unpredictable and highly dynamic conditions of an agricultural grassland (mowing, fertilizing several times each year). Good colonizers such as phoretics having a short development time become more abundant. In fig. 1 fractions of life-history tactics of mites and springtails from the microarthropod community in two grasslands are presented. The presented life-history tactics are an elaboration of those established by Siepel & Van de Bund (1986). Both grasslands are on the same soil type,

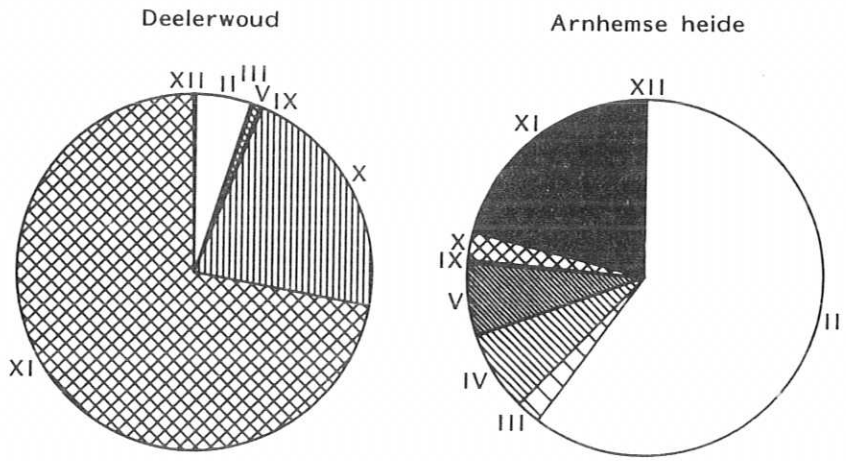

Fig. 1. Fractions of life-history tactics of microarthropods in an agricultural grassland "Arnhemse heide" and a heathy natural grassland "Deelerwoud". Characterization of tactics: II (faculative phoretic), III (obligate juvenile phoretic), IV (obligate phoretic mainly as adult), V (obligate diapause), IX (thelytoky and seasonal iteroparity), X (thelytoky), XI (sexual reproduction) and XII (sexual reproduction and seasonal iteroparity).

a typic haplohumod, but "Arnhemse heide" has become an agricultural grassland during the last five decades whereas "Deelerwoud" remained a heathy natural grassland. The differences are very clear: tactics II (facultative phoresy), III (obligate juvenile phoresy), and IV (obligate phoresy mainly as adult) are more abundant in the agricultural grassland Arnhemse heide than in the natural grassland Deelerwoud. Tactics IX (thelytokous reproduction and seasonal iteroparity) and X (thelytokous reproduction) are more abundant in the natural grassland Deelerwoud than in the agricultural grassland Arnhemse heide. Species of tactic XI are sexually reproducing and are not phoretic. The presented differences are understandable; phoretic species are better adapted to sudden changes in their biotope, as a population they can easily turn aside for a while and colonize the grassland again later. Species from tactics IX and X (thelytokous species) on the contrary have no phoresy by definition and a way of reproduction adapted to very constant and predictable environments. The daughters inherit the same genotype as their mother had, which is successful only under the same, constant, conditions. Rapid colonization in those species will not occur.

THE FUNCTION OF THE MESOFAUNA IN DECOMPOSITION OF ORGANIC MATTER

Differences in species composition of mite and springtail communities among natural and agricultural grasslands can be explained by their life-history tactics. The question now is how do the different communities affect the process of decomposition of organic matter. When all mite and springtail species act roughly the same on the rate of decomposition, there will hardly be any change in effect. However, that these species all affect the process of decomposition in the same way is quite unlikely and has been found incorrect as appears in table 1. Already among oribatid mites only several feeding guilds can be established. The five most common ones with their definition based on carbohydrase enzym activities and a species example are presented in table 1. The feeding guilds are defined by the activity of three important carbohydrase enzyms, that differentiate in the used food types. Trehalase activity means ability to digest trehalose, a sugar occurring predominantly in fungi (cell contents), chitinase activity means ability to digest chitin, occuring, next to the exosceleton of many arthropods, predominantly in fungi (cell walls) and finally cellulase activity

Table 1. Five different feeding guilds defined by their activity of carbohydrases are listed with their specifications (from Siepel & De Ruiter-Dijkman) and their influence on the rate of decomposition of organic matter, ch = chitinase activity, tr = trehalase activity, ce = cellulase activity, eff = effect on decomposition rate.

	ch	tr	ce	eff	
fungivorous grazer	+	+	-	+	*Punctoribates punctum*
herbofungivorous grazer	+	+	+	+	*Nothrus silvestris*
herbivorous grazer	-	-	+	0	*Parachipteria punctata*
opportunistic herbofungivore	-	+	+	-	*Carabodes labyrinthicus*
fungivorous browser	-	+	-	-	*Chamobates borealis*

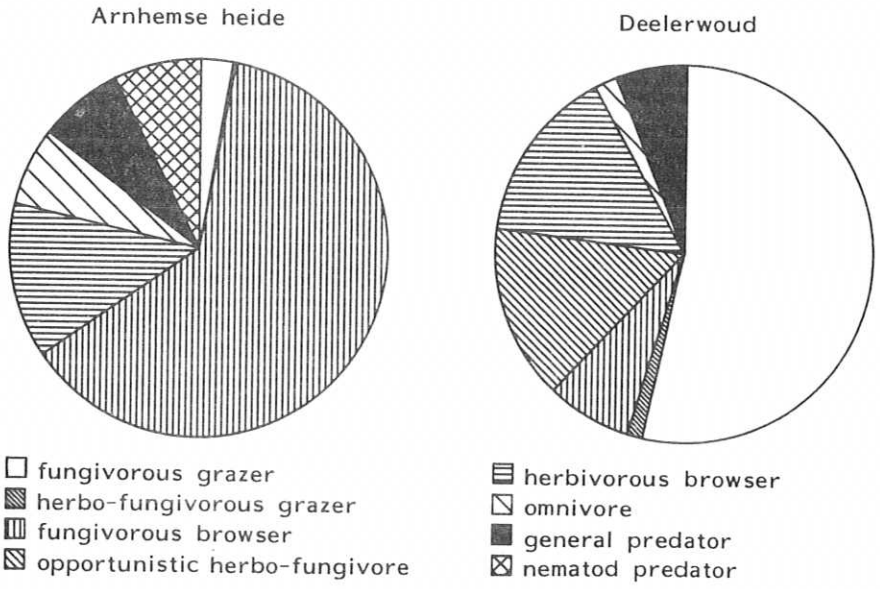

Fig. 2. Fractions of feeding guilds of microarthropods in an agricultural grassland "Arnhemse heide" and a natural heathy grassland "Deelerwoud". See table 1 for definition of the main not predatory feeding guilds.

means ability to digest cellulose, an important cell wall component of plants. According to table 1 fungivorous species, able to break down chitin (fungivorous grazers and herbofungivorous grazers), have a stimulating effect on the rate of decomposition. Species that do not feed on fungi (herbivorous grazers) have no effect at all and species feeding on fungi but digesting only the fungal cell contents (oppurtunistic herbofungivores and fungivorous browsers) have a inhibitory effect on the decomposition rate. Explanation of these differences lies in the impact of grazing of mites on the fungi and in the recycling of scarce elements. The impact of grazing is greater when a mite species retrieves its energy from cell contents of fungi only compared to one retrieving its energy from cell contents and cell walls as well. So opportunistic herbofungivores and fungivorous browsers will overgraze fungi sooner than fungivorous and herbofungivorous grazers do under the same mite to substrate mass ratio. (Differences in size and energy requirements in mites have been corrected for by carrying out experiments with the same total mass of mites.) Overgrazing has been recorded by Hanlon & Anderson (1979) before as inhibiting. The stimulating effect of fungivorous and herbofungivorous grazers, can be explained by the recycling of scarce elements.

Most probable is nitrogen: it makes up 6.9 % by mass of chitin, which can form up to 25% of cell wall mass of fungi. So an extra mass of up to 1.7% nitrogen from fungal cell walls is recycled by chitin digesting mites compared to mites without that chitin digesting ability. Under conditions of nutrient impoverishment which goes with restoration of natural processes and communities, this extra mass of recycling nitrogen may be of crucial importance in a well functioning of the decomposition process, especially when the change in the plant community from species having low C/N ratios to those with higher ones also takes place during the process of nutrient impoverishment. When the microarthropod species cannot reach the natural grassland in view, the process of nutrient impoverishment in grasslands on slightly acid sandy soils will result in the comparatively malfunctioning of the decomposition process and thus accumulation of litter and felting of the grassland vegetation. A situation in which germination of seeds from seed banks or from invading plant species is strongly inhibited.

DISCUSSION

Restoration of natural processes and communities in abandoned agricultural areas may not be achieved by just some nutrient impoverishment as one would wish. Decades of agricultural management have changed the soil and soil fauna tremendously and it might be a bit naive to suppose that restoration will take place just as is wished. I present in this paper not the solution of a detected problem of felting and the accumulation during nature restoration. I try to make acceptable that the mesofauna has an important and until now overlooked role in nature restoration, that this decomposer role is influenced by agricultural management, resulting in a change of the soil microarthropod species composition, and that it is unlikely without additional measures, to expect a functioning microarthropod community as existed before agricultural disturbance. However, theoretically acceptable, practice should prove whether or not the microarthropod community is the key factor in the problem of litter accumulation and felting of the grassland vegetation during the process of restoration of natural communities and processes. Man has caused the shift from a natural to an agricultural grassland microarthropod community and so it is not strange that man restores artificially the proper soil fauna. It can be done by grafting the abandoned agricultural area with soil and litter, including the mesofauna, from natural stands in the form of compost resulting from sod cutting in grassy heathlands. This possible solution should first be carried out on a small scale. As I have mentioned here, it has a good chance of success. When it has turned out ot be a realistic and workable solution, a major problem in the restoration of natural processes and communities can be overcome.

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THE INFLUENCE OF CATTLE GRAZING INTENSITY ON GRASSHOPPER ABUNDANCE (ORTHOPTERA: ACRIDIDAE)

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Keywords:
Acrididae, grazing, vegetation structure, nature management.

Summary

This study deals with the effects of grazing on grasshoppers with respect to nature management. Positive and neutral effects of grazing on grasshopper abundance compared to ungrazed grasslands were found; intensive grazing had negative effects compared to extensive grazing. These diverging effects are explained by a model in which the relationship between grasshopper abundance and the amount of vegetation left over by grazing follows an optimum curve. Underneath excessive vegetation egg development is hindered whereas at conditions of shortage of vegetation structure the shelter for nymphs and adults is lost.

INTRODUCTION

Increasing atmospheric nitrogen deposition and other forms of eutrophication necessitate intensification of management rules aiming at nutrient impoverishment in nature reserves. Nowadays application of grazing occurs more and more frequently because of its relative cheapness and its supposed positive effect on biodiversity.

Several comparisons of the effects of grazing on arthropod abundance with the effects of mowing and no management turned out to be unfavourable for grazing (see for review: Siepel et al., 1989). Concerning grasshoppers the intensity with which grazing is applied, is crucial and in addition has opposite effects on different systematic groups (Capinera & Sechrist, 1982). In order to obtain more detailed information on the effects of grazing intensity on grasshoppers we have studied their abundance in three types of nature reserve grassland which are subjected to grazing frequently, *viz.* *Deschampsia flexuosa* (L.) Trin. and *Molinia caerulea* (L.) Moench dominated heathland, and former agricultural grassland. The expression of grazing intensity in head of cattle.area-1 is not a relevant measure for field layer insects. Therefore grasshopper density is related to grazing intensity through the parameters light extinction by the vegetation and vegetation height, as well as the horizontal heterogeneity of these variables.

MATERIAL AND METHODS

Data on the study areas are summarized in Table 3. Unless indicated else grasshopper densities have been estimated by means of

*Table 1. Grasshopper numbers in ungrazed and grazed Deschampsia dominated plots (Wolfhezerhei) in three subsequent years. a. Number of plots (n plots); means and standard errors ( ) of number of species (n spec); idem, of individuals of all species (N); b. idem, of S. stigmaticus (N stig); idem, of M. maculatus (N mac); idem, of other species (N others). Pitf.:numbers sampled by means of pitfall trapping; data from Oova & Van Steenis (1988), by courtesy of Ir. J. Bokdam; rem.: densities estimated by means of removal trapping (N.are-1). c. Results of statistical testing (GLM, F-test, P = probability; * : < 0.05; ** : < 0.01; *** < 0.001; n.s. : > 0.05).*

	ungrazed			grazed		
a. year/meth.	n plots	n spec	N	n plots	n spec	N
1987/pitf.	2	2.5(0.7)	18.5(15.6)	4	3.5(0.6)	510.0(57.9)
1988/rem.	3	0.3(0.2)	0.7(2.4)	3	1.7(0.5)	63.7(23.6)
1989/rem.	4	1.5(0.4)	6.3(6.4)	2	2.5(0.7)	41.5(23.4)
b.	N stig	N mac	N others	N stig	N mac	N others
1987/pitf.	6.5(7.2)	11.5(14.3)	0.5(0.6)	435.3(41.7)	72.3(25.4)	2.5(0.9)
1988/rem.	0.7(1.9)	0 (0.1)	0 (0)	61.7(18.1)	2.0(4.9)	0 (0)
1989/rem.	5.8(4.8)	0 (0.1)	0.5(0.4)	40.0(17.9)	0.5(3.0)	1.0(0.8)

c. effects

grazing on:	F	P
n spec	6.3	*
N	63.3	***
N stigm	97.8	***
N mac	3.6	n.s.
N others	2.8	n.s.
year/method on:		
all variates	>5.1	*
interaction on:		
all variates	<1.5	n.s.

Table 2. Grasshopper numbers in two subsequent years in three meadows (Bovenbuurt) differing in grazing intensity (head of cattle per ha). a. Number of plots (n), means and standard errors ( ) of number of species (n spec); idem, of number of individuals of all species (N); b. idem, of C. paralellus (N para); idem, of C. albomarginatus (N albo). c. Results of statistical analysis.

Grazing intensity	2.3			3.6			4.9		
a. year	n	n spec	N	n	n spec	N	n	n spec	N
1987	3	2.0(0.5)	141.4(39.1)	2	1.5(0.6)	2.4(6.2)	2	1.5(0.6)	2.8(6.7)
1988	3	2.0(0.5)	14.7(12.6)	3	1.7(0.5)	3.2(5.9)	3	1.3(0.4)	1.8(4.5)
b.		N para	N albo		N para	N albo		N para	N albo
1987	3	38.7(13.5)	102.7(26.3)	2	0.5(1.9)	1.9(4.3)	2	1.0(2.7)	1.8(4.2)
1988	3	8.7(6.4)	6.0(6.4)	3	1.7(2.8)	1.5(3.2)	3	0.5(1.5)	1.3(3.0)

c. effects

grazing on:	F	P
n spec	0.7	n.s.
N	9.9	**
N para	6.9	*
N albo	11.3	**
year on:		
n spec, N para	<3.9	n.s.
N, N albo	>10.2	**
interaction on:		
all variates	<0.5	n.s.

removal trapping within fenced plots of 100 or 200 m2 which were representative and comparable. Vegetation structure has been quantified by calculating the light radiation extinction from measurements at the soil surface level and over the vegetation. Vegetation height has been measured with the help of a tempex plate (Ø = 0.5 m; 390 g) which has been let down on the vegetation along a vertical rod with scale graduation. Means and coefficients of variation have been calculated from 25 measurements in a regular grid pattern.

The statistical theory of Generalized Linear Models (GLM) (Dobson, 1983) has been used for estimating the effects of grazing on numbers and for testing of the differences. As link function the log function has been used, so that the models of expectation are multiplicative models. The most general model (alternative hypothesis) is the one with both main effects and (multiplicative) interaction. Interaction has been tested against this model. Both main effects have been tested assuming that there are no interactions, but the other main effect may differ from zero. The tests have been done with approximated F-tests using the deviance of the full model as second deviance. Dispersion has been estimated before by dividing the deviance of the full model by its number of degrees of freedom, under the assumption of Poisson distributions.

Linear regression analysis has been used for estimating regression coefficients, intercepts and percentages of variance accounted for.

*Table 3. Data on study areas. Head : head of cattle.ha-1; * : young cattle; ** : additional rabbit grazing; *** : only sampling area Schotse Heide is considered.*

study area	management	head	area	duration	since	intensity	municipal.
Wolfhezerhei	grazed	0.3	60 ha	year	1983	intensive	Renkum
	ungrazed		54				
Imbos***	grazed	0.2	30	year	1982	intensive	Rozendaal
Deelerwoud	ungrazed		>100				Ede
Needse Achterveld	grazed	0.1	68	year	1983	extensive	Neede
	ungrazed		5				
Bovenbuurt	grazed	4.9*	2.2	summer	1985	intensive	Wageningen
	grazed	3.6*	1.9	summer	1971	intensive	
	grazed	2.3*	2.2	summer	1985	extensive	
Junner Koeland	grazed	1.0*	50	summer	1967	extensive	Junne
	grazed	1.0**	50	summer	1967	intensive	

RESULTS AND DISCUSSION

Grazing is classified here as either extensive or intensive. Sample areas are called extensively grazed when less vegetation is consumed than produced annually so that pieces of high and dense vegetation are left over. Grazing is described as intensive when at a certain moment in the year consumption equals production in the sample area; with respect to grasshoppers the latter grazing regime can be extensive if this moment falls beyond the lifetime of grasshopper nymphs and imagines (e.g. Wolfhezerhei).

Table 4. Results of regression analyses of grasshopper density on mean light radiation extinction, mean vegetation height and coefficients of variation (CV). t : values used in Student's t-test for testing significance of correlation coefficient; for P, see Table 1; PVA : percentage of variance accounted for by the independent variable. Deschampsia plots were situated in the Wolfhezerhei, Deelerwoud and Imbos areas.

	regression equation	t	P	PVA
density *S. stigmaticus* in *D. flexuosa* (1989)				
12 (un-)grazed plots:	ln N = 5.44 - 1.14 extinction	-4.8	***	66.8%
idem	ln N = 2.32 + 0.01 CV extinction	0.4	n.s.	
7 ungrazed plots:	ln N = 6.54 - 1.47 extinction	-3.4	*	63.3%
overall and species densities in Bovenbuurt meadows (1988)				
overall density:	ln N = 0.62 + 1.13 extinction	4.4	*	69.4%
idem	ln N = 1.35 + 0.00 CV extinction	0.2	n.s.	
idem	ln N = -0.97 + 0.33 height	7.2	**	86.3%
idem	ln N = 0.50 + 0.03 CV height	0.6	n.s.	
C. parallelus:	ln N = 0.20 + 1.06 extinction	4.1	*	66.7%
C. albomarginatus:	ln N = 0.47 + 0.80 extinction	5.2	**	76.6%

- First data set: intensive grazing of *Deschampsia* dominated heathland

In the Wolfhezerhei area the species number as well as the overall density of grasshoppers of plots being grazed during four years were significantly larger than those of the ungrazed plots (Table 1). The largest contribution to this difference was accounted for by *Stenobothrus stigmaticus* (Rambur) which was significantly more abundant on the grazed plots. This, however, was not the case with the numbers of *Myrmeleotettix maculatus* (Thunberg) and those of the other species, viz. *Omocestus viridulus* (Linnaeus), *Stenobothrus lineatus* (Panzer), *Metrioptera brachyptera* (Linnaeus) and *Chorthippus parallelus* (Zetterstedt), together. The contribution of the latter species group to overall density was small. The differences between years of sampling were caused by the considerably greater numbers captured by means of pitfall trapping compared to removal trapping. There were no significant interaction effects (Table 1).

The density of *S. stigmaticus* and the mean light extinction at the soil surface level were correlated negatively, for the complete set of 12 grazed and ungrazed plots as well as for 7 ungrazed plots (Table 4). The latter correlation indicates that differences in abundance between grazed and ungrazed plots, too, originate from differences in vegetation structure rather than from other effects of grazing.

Table 5. Means and standard errors ( ) of species number, overall density and species density (N.are-1) in three ungrazed and eight grazed Molinia dominated plots in the Needse Achterveld area. For F and P, see Table 1.

	ungrazed	grazed	F	P
Number of species	4.0(0.4)	3.8(0.2)	0.3	n.s.
Overall density	45.0(12.5)	45.0(7.7)	0.0	n.s.
M. brachyptera	29.0(7.4)	18.3(3.6)	2.0	n.s.
C. parallelus	8.0(5.0)	14.5(4.1)	0.9	n.s.
M. grossus	6.3(3.8)	4.0(1.9)	0.4	n.s.
O. viridulus	1.7(1.9)	6.5(2.3)	1.9	n.s.
M. maculatus	0.0(0.0)	1.8(1.1)	1.6	n.s.

- Second data set: extensive grazing of *Molinia* dominated heathland

After five years of very extensive grazing there were no significant differences between the grazed and the ungrazed part of the Needse Achterveld in overall grasshopper density, species number as well as the densities of the separate species (Table 5). Here the extinction of light radiation at the soil surface level by the vegetation could not be measured with confidence with our routine procedure due to both the structural density and the elevated position of the *M. caerulea* tufts.

- Third data set: extensive and intensive grazing of former agricultural meadows

After three years of grazing with different numbers of head of cattle in the Bovenbuurt area grazing intensity influenced the overall grasshopper density significantly; in the extensively grazed plots larger densities occurred (Table 2). This also applied for the densities of each of both separate species, *C. parallelus* and *Chorthippus albomarginatus* (Degeer), but not for the species number. Furthermore there was a significant effect of the year of sampling on the density of *C. albomarginatus* as well as on the overall grasshopper density; this effect may have been caused by early adult mortality due to relatively cold and wet weather during the weeks prior to sampling in 1988. Interaction effects between grazing intensity and year of sampling did not exist (Table 2).

The obvious negative effect of high grazing pressure on grasshoppers has been confirmed by results from the Junner Koeland area. In grassland plots which were shortly grazed ("trimmed") over the whole area due to cattle and rabbit grazing significantly less grasshoppers were found than in plots being grazed more extensively by cattle only and which therefore had a higher vegetation (Table 6).

The overall grasshopper density and the mean light extinction at the soil surface in the Bovenbuurt plots were correlated positively. This also applied for each of both separate species (Table 4).

Table 6. Means and standard errors ( ) of light radiation extinction and coefficients of variation, species number, overall density and species densities (N.are-1) in three plots which were extensively grazed by cattle, and three plots which were in addition (intensively) grazed by rabbits in the Junner Koeland area. For F and P, see Table 1.

	extensive	intensive	F	P
mean extinction	0.90(0.08)	0.19(0.03)	69.6	**
coeff. variation	49.0(7.1)	71.6(8.6)	4.2	n.s.
overall density	181.3(63.5)	1.7(6.1)	10.6	*
species number	4.0(1.5)	1.0(0.7)	3.5	n.s.
C. parallelus	146.3(47.5)	0.7(3.2)	12.6	*
S. stigmaticus	23.3(11.2)	0.7(1.9)	5.1	n.s.
O. viridulus	8.0(3.9)	0.3(0.8)	4.7	n.s.
C. biguttulus	3.3(1.8)	0.0(0.0)	4.6	n.s.
M. maculatus	0.3(0.2)	0.0(0.0)	2.5	n.s.

- A model for the influence of cattle grazing

The correlations between grasshopper density and mean light extinction (Table 4) indicate that grazing intensity acts on

grasshoppers through its influence on the quantity of vegetation left over. From this as well as from the absence of a correlation with the coefficients of variation of extinction and height (Table 4) it can be concluded that the presence of high (and dense) vegetation *per se* is more important than the occurrence of a great variation in extinction and height.

The positive (first data set) and more or less neutral (second set) effects of grazing as well as the negative (third set) effects of intensive grazing can be understood with the help of a conceptual model in which the relationship between grasshopper abundance and the amount of vegetation left over by grazing follows an optimum curve (Fig. 1). This amount is negatively correlated to grazing intensity but positively to the production level of the grassland. Starting off from high and dense vegetation on nutrient-rich soil (e.g. first data set; right tail of the model) grazing brings about shortly grazed spots (Fig. 1). As long as the remaining parts with high and dense vegetation will not become too scarce, grasshopper density will increase. But if the distance between such parts (in relation to grasshopper ranges) becomes too large (e.g. intensively grazed plots of the third data set; left tail of the model) grasshopper density will fall. In grasslands with a less luxuriant vegetation the right tail of the model (Fig. 1) will be cut off. Starting off extensive grazing in such vegetation will have either neutral (second data set) or small positive effects, which will depend on the nearness to the optimum. The model predicts that a relative small enlargement of the grazing pressure may have negative effects. The model, however, is provisional; the next step is to verify it for the separate species.

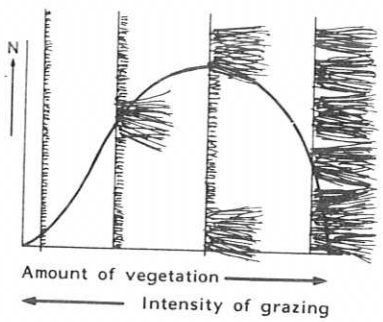

Fig. 1. Visualization of a conceptual model describing the relationship between grasshopper abundance and the amount of vegetation left over by grazing as well as grazing intensity starting off from a high and dense vegetation on nutrient-rich soil.

- Causal factors: opposite demands of different life stages

At conditions of excess vegetation (right tail of the model; Fig. 1) egg development is hindered by the strong extinction of light radiation at the soil surface (on or under which the eggs are deposited). As maximum temperature near the soil surface is strongly influenced by radiation extinction (Barkman & Stoutjesdijk, 1987) and egg development rates increase strongly with increasing temperature (Van Wingerden *et al.*, in prep.), egg development rates will be low underneath high and dense vegetation. Consequently grasshopper species, which have a relatively long egg development duration, may not or hardly be able to accomplish their lifecycle in such a vegetation before winter, and will therefore either be absent or occurring in low numbers. A second negative effect of high and dense

vegetations may be that grasshoppers are unable to display specific behaviour which is suggested for *M. maculatus* by Sänger (1977). Thirdly, the edible parts of the vegetation may be hard to find, especially the parts which are rich in nutrients; this could cause a low feeding efficiency.

At the other hand a high and dense vegetation has also strong positive effects. It offers shelter to grasshopper nymphs and imagines against rain, wind and intensive sun radiation (Lensink, 1963); in addition it reduces extreme fluctuations in temperature (Bossenbroek, 1977) and moisture. This means that conditions of shortage of high and dense vegetation (left tail of the model; Fig. 1) most probably result in enhanced dispersal or mortality of grasshoppers.

So it can be concluded that the egg stage at the one hand and the nymphal and imaginal stages at the other hand may have opposite demands on vegetation structure. From this it can be inferred that large grasshopper abundances (optimum of the model; Fig. 1) may occur in grasslands with (pieces of) low vegetation during egg development (May - June) and pieces of high and dense vegetation during the lifetime of nymphs and imagines (June - September).

Although further evaluation of the model and the causal factors is needed, we expect that the presented causal-analytical approach will contribute to the discussion on application of grazing in nature reserves.

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IS THE ALLERGEN LEVEL OF HOUSE DUST RELATED TO AGE OF THE HOUSE?

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Keywords:
House dust mites, *Dermatophagoides spp.*, allergens, house age.

SUMMARY

House dust mites *Dermatophagoides* species (Acari: Pyroglyphidae) produce allergens, known for the provocation of asthma and other allergic reactions.

To determine the time needed for the complete colonisation of a new home by house dust mites, dust samples were collected from carpets of houses varying from 2 weeks to 2 years in age. In contrast to the expectation, no relation was found between average levels of the allergens Der pI and Der pII per m^2 and age of the houses.

However, a positive relation was revealed between the presence of dogs and the number of occupants in the house on one hand and allergen levels on the other hand. Furthermore, carpets in bedrooms appeared to contain more allergens than carpets in living-rooms.

Finally, the age of the mattress was not related to allergen levels of bedroom floors.

INTRODUCTION

Since the work of Voorhorst et al. (1969), it is generally accepted that house dust mites produce allergens. Their faeces are highly allergenic (Tovey et al., 1981) and can provoke asthma and other allergic reactions when inhaled (Wharton, 1976). Especially *Dermatophagoides pteronyssinus* (Trouessart), *D. farina* Hughes and *D. microceras* Griffiths and Cunnington are known for their allergenic properties (Heymann et al., 1989).

D. pteronyssinus and *D. farinae* are cosmopolitan species. *D. pteronyssinus*, more susceptible to desiccation than *D. farinae*, is extremely common in house dust in Western Europe (Van Bronswijk, 1981). Mite numbers show an annual periodicity, which coincides with the annual humidity cycle in the house (Spieksma, 1967). House dust mite populations are at peak levels in late summer (Blythe, 1976) or autumn when relative humidity is usually highest (Van Bronswijk & Sinha, 1971), and lowest in spring (Blythe, 1976). In Ohio (USA), for example, the average numbers of live mites per gram dust were more than 10 to even more than 25 times higher in late summer than in spring (Arlian, 1982). A Dutch study revealed that the allergen level of house dust was on average only 5 times lower in spring than in autumn (Van Leeuwen & Aalberse, 1988). Apparently, decomposition of allergens is a slow process.

The highest numbers of mites are found in places where dust (e.g. skin scales), the food source for house dust mites, accumulates, such as beds, overstuffed furniture

and carpets (Arlian, 1989). On the other hand, no house dust mites were detected on floors of new houses until human inhabitants had used their home for one week (Van Bronswijk (1974), in The Netherlands) or for one month (Miyamoto & Ouchi (1976), in Japan). Arlian (1982) came across one home (out of 26) without any mites. This house was less than two months old and contained new furnishing and carpeting. So far, no information is available on the length of time needed for complete colonisation of a new home by house dust mites.

The object of the present study is to assess the relation between allergen levels of house dust collected from the carpet and the occupation time of the home. In brand new homes with new carpets a very low allergen level is expected, since there will not be enough food to allow fast mite population growth. Furthermore, an increase in mite numbers with increasing occupation time is expected until the carrying capacity is reached.

MATERIAL AND METHODS

Dust samples

Dust samples were collected in bedrooms and living-rooms in the second half of May 1990. The age of the homes varied from 2 weeks to 2 years. Only rooms with new carpets at the start of occupation were sampled. A total of 114 rooms in 60 different houses in Dronten (Flevoland, The Netherlands) was sampled.

Information on various parameters which might influence mite numbers was obtained by questioning the residents. Questions concerned e.g. the number of occupants and pets in the house, carpet material, age of mattress and the habit of eating in the bedroom.

Samples were taken using a Moulinex® Compact 1250 Vario Electronic vacuum cleaner at 800 Watt. This vacuum cleaner was equipped with a built-in sampling device in a specially constructed hose. The sample was collected in a glass tube with an inner diameter of 4.3 cm, which was covered at one end with a filter cut out of the densest layer of double layer Moulinex® dust bags.

In order to collect one sample, 2 m^2 of the carpet were vacuum cleaned for 4 minutes. Relative humidity and temperature were recorded at ground level in the sampled room at the time of sampling. After taking one sample, the glass tube with the sample was replaced by a new one. Parts of the vacuum cleaner which had been in contact with the just collected sample were either replaced or cleaned. All samples were taken by the same person.

The tubes containing the samples were stored, for a maximum of two days, in separate plastic bags at 4 °C until the samples were weighed. Part of each sample was stored at -20 °C until, a few weeks later, allergen assessments were done.

Allergen assessments

Measurements of the allergens Der pI and Der pII were done at the Department of Immunochemistry of the Central Laboratory of The Netherlands Blood Transfusion Service. Immunochemical techniques were applied, i.e. two different radio-immuno-assays (RIA's) were used to quantify the respective allergen activity. The antibodies of the RIA applied for determination of Der pI respond quite specifically to group I allergens of *D. pteronyssinus*, while the antibodies of the second RIA do not distinguish between group II allergens of the three *Dermatophagoides* species (Van der Zee et al., 1988; Van Leeuwen & Aalberse 1988).

Statistical analysis

Student's *t*-test and Analysis of Variance were applied on log transformed data to analyse the results.

Some samples were excluded from further (statistical) analysis because both allergen levels were below or above the detection level of the test. In case of one allergen of a sample exceeding a test limit, the results of the other allergen were processed as usual.

RESULTS

No relation was found between allergen levels or dust quantity from the carpet and the age of a house (Figure 1).

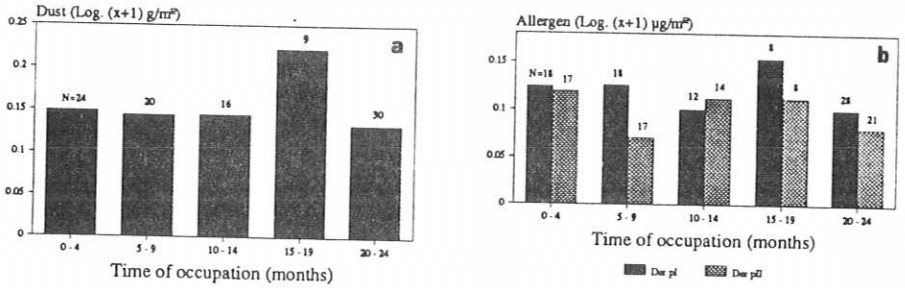

Figure 1. *Average dust quantity per m²* (a) *and average level of the allergens Der pI and Der pII per m²* (b) *in relation to the occupation time of the house.*

Figure 2a shows the dust quantity per m² in presence or absence of dog or cat in the household. There is no significant difference between households without a dog or cat and households with a cat only. However, the presence of a dog is associated with a higher average dust mass ($P < 0.01$), both compared to households without a dog or cat and households with a cat. (The three data concerning households with both a cat and a dog were left out of this analysis.)

The average level of both allergens per m² differed significantly ($P < 0.01$), between households without a dog or cat and those with just a dog (Figure 2b). (Data regarding households with either both a cat and a dog or a cat only were excluded because of too small sample sizes.)

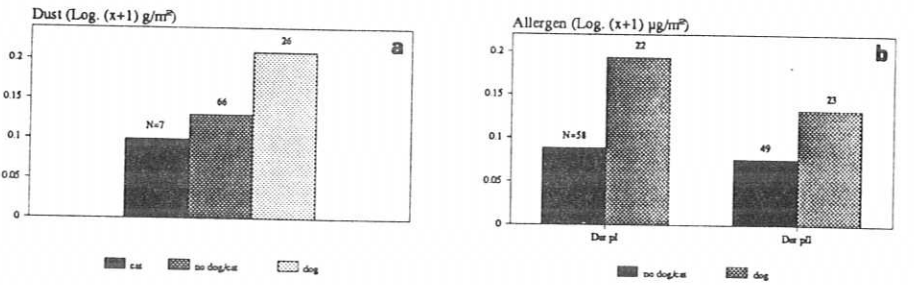

Figure 2. (a) *Average dust quantity per m² in absence or presence of cat or dog.* (b) *Average level of the allergens Der pI and Der pII per m² in households without a cat or a dog and those with a dog present.*

No significant differences in dust quantity or allergen levels were found between single and double bedrooms. On the other hand both the average dust quantity and average allergen levels were higher ($P < 0.01$) in bedrooms than in living-rooms (Figure 3).

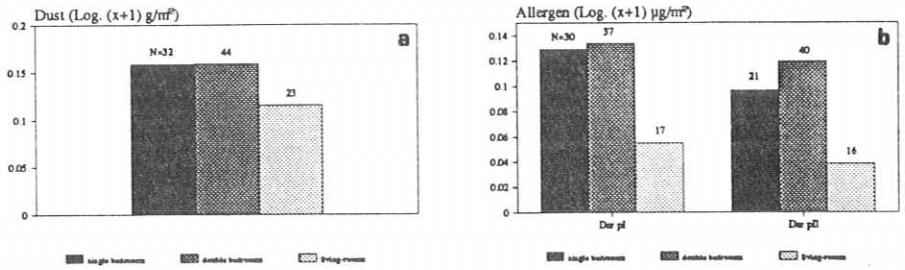

Figure 3. *Average dust quantity per m²* (a) *and average level of the allergens Der pI and Der pII per m²* (b) *in single and double bedrooms and in living-rooms.*

No significant difference was found between average dust quantities of living-room carpets in houses with one or two occupants and those with three, four or five occupants (Figure 4a). However, there is a trend ($P < 0.05$) towards a higher average level of Der pI per m^2 in larger households. As holds for the dust quantities, there is no significant difference between the level of Der pII per m^2 when the two categories of households were compared (Figure 4b).

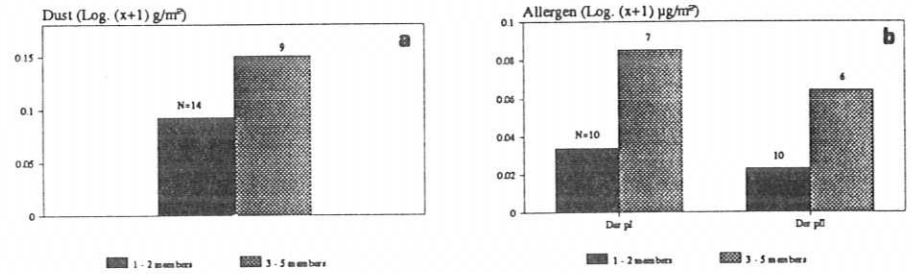

Figure 4. *Average dust quantity per m²* (a) *and average level of the allergens Der pI and Der pII per m²* (b) *in living-rooms of houses in relation to the number of occupants in the house.*

No relation was found between the age of the mattress or eating in the bedroom on one hand and dust quantity or allergen levels per m^2 on the other hand.

DISCUSSION

The results presented here are based on a preliminary analysis of the data. For instance, possible interactions of factors have not yet been studied. Moreover, the possible influence of factors such as temperature and relative humidity (at the moment of sampling) have not yet been taken into consideration. The same applies to, for instance, (approximate) age of the occupant(s). Additionally, it is necessary to check if factors of proven influence, such as the presence of dogs, are evenly

distributed over the classes of occupation time.

In contrast with the expectation, allergen levels and occupation time of the houses were not related (Figure 1). A possible explanation is that allergens in very new homes are mainly brought in, for instance with overstuffed furniture or blankets, and are not produced on the spot. Importation of allergens with blankets is not unlikely since Sesay & Dobson (1972) found blankets to contain even higher mite numbers than mattresses. Another possibility is that carpets themselves serve as a food source. In this case food is, from the very moment of occupation never a limiting factor; mite population increase is possible.

However, more striking are the very low allergen levels, sometimes even lower than the detection level of the tests, in some 'older', i.e. almost two years old houses. To determine the factors involved here might even be more important, since they could provide a key for the prevention of high population levels of house dust mites.

The presence of a dog in households is related to a higher dust quantity as well as higher allergen levels (Figure 2). Of course, this does not necessarily mean that the dog itself is the cause of these differences; presence of a dog might be related to other factors. However, a dog, like human beings, produces skin scales and human skin scales are known to be a good food source for house dust mites (Spieksma, 1967).

Arlian (1982) did not find mite abundance to be correlated with the presence or absence of pets. Hart & Whitehead (1990) came to the same conclusion after analysis of the mite fauna of living-room carpets; no significant relation was found between total Pyroglyphid mite numbers and pets' beds and dining areas or the number of pets in the house. It is not clear if these two authors distinguished between different pets, such as cats and dogs. For example, Figure 2a shows that cats are possibly associated with an opposite effect on the dust quantity compared to that of dogs. Comparison of allergen levels in households with either a cat or a dog could not be made here, because of too small sample sizes in the 'cat groups'. However, it is possible that cats and dogs have opposing effects on dust mite populations.

Dust quantity and allergen levels were higher in bedroom carpets than in carpets of living-rooms (Figure 3). The latter is in accordance with the findings of Blythe (1976) that living-room carpets usually contain fewer mites than those in bedrooms. Blythe assumed that this difference is due to different cleaning routines or the scattering of mites from bedding. Further analysis of the data will indicate if different cleaning routines are a possible explanation in this case. Mulla et al. (1975) concluded that there is a tendency for heavier infestations to occur in bedrooms with more occupants. This cannot be confirmed in the present study, but Mulla et al. sampled bedrooms with up to four occupants, while in the present study the maximum number of occupants was two.

No significant relation was found between household size and dust quantity or Der pII level ($P = 0.06$, in both cases). However, larger households were associated with a higher Der pI level than smaller households (Figure 4). This is in accordance with the findings of Arlian et al. (1978), who showed that average mite infestations were proportional to the number of members in the household. A likely explanation is that in larger households both more skin scales and moisture are produced. Additionally, more human food might be spilled on the floor, since larger houshold size usually implies the presence of one or a few (small) children. Furthermore, in larger households there might not only be an increase in the intensity of use, but also

in the duration, since on average more time/day/person is spent at home. If this is true, the effect of household size could even be stronger.

Whether any of the above speculations are supported by the data presented here, will be the subject of further statistical analysis, to be published elsewhere.

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LIRIOMYZA HUIDOBRENSIS (BLANCHARD) (DIPTERA: AGROMYZIDAE) A NEW ECONOMICALLY IMPORTANT LEAF MINER IN THE NETHERLANDS

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Keywords:
Leaf miner, horticulture, economically important measures.

ABSTRACT

Since the summer of 1989 the American leaf miner *Liriomyza huidobrensis*. has caused a great deal of damage in several horticultural crops in the Westland region of the Netherlands. These difficult to characterize *Liriomyza spp.* were identified on the basis of morphology and by protein electrophoresis. A national inventory has provided a better insight into the spread and behaviour of this pest in the Netherlands. Effective information and intensive control have resulted in a controllable situation.

INTRODUCTION

In the summer of 1989 the American leaf miner *Liriomyza huidobrensis* (Blanchard) was identified as the cause of considerable economic damage in Dutch horticulture. In the Westland region, problems arose in cultivation of (iceberg) lettuce, gypsophila and tomato, which were different from other well known affects of insects. Many lettuce had to be ploughed in because they were no longer saleable.

The damage was the result of different factors. Since the larvae made most of their tunnels on the underside of the leaf the attacks were not discovered until large populations had built up. Resistance existed to different chemical compounds. Moreover, as far as the quick growing vegetable crops are concerned, there are obviously limitations where the use of chemicals are concerned. The unusual warm spring of 1989 and the summerlike temperatures continuing far into the autumn also aided rapid and successful development of the successive leaf miner generations.

After this new leaf miner was identified (this was later confirmed by the leaf miner specialist, K.A. Spencer) biological characteristics were looked up in the literature. This species is especially polyphagous and is known to cause damage in a large number of crops on the American continent where it

originated (Spencer. 1980. Parrella. 1982). Considerable damage results from tunneling by the larvae as well as by the many puncture holes made in the leaves by the ♀♀ feeding before oviposition.The damage caused by an extensive attack of *L. huidobrensis* is greater than that by an earlier American immigrant *L. trifolii*. Damage to the spongy mesophyll caused by *L. huidobrensis* leads to a greater reduction in photosynthesis than when the palisade mesophyll is attacked by *L. trifolii* (Parella et al.. 1985). Resistance to different insecticides makes chemical control difficult (Raman. 1988). For these reasons *L. huidobrensis* is one of the quarantine species for the European Economic Community (EEC) and the European and Mediterranean Plant Protection Organization (EPPO)-areas (Anonymous. 1984). entry being refused to any infected material.

The Plant Protection Service has taken steps to bring this leaf miner under control and stop its further spread and research institutes have also been brought in to develop an effective method of control. It is important that cultivation and export should not be allowed to stagnate.

MATERIALS AND METHODS

After establishing the presence of *L. huidobrensis* in the Netherlands the Plant Protection Service started an inventory of its distribution and crops affected. A reliable and preferably quick method of identification was therefore necessary. This species is difficult to separate morphologically from *Liriomyza bryoniae* (Kalt.). the tomato leaf miner. which is commonly found in Europe and is biologically held under control in the Netherlands. Differences in colour and morphological differences in the sex organs can be used for identification. An exact characterization on the basis of morphological characteristics of the pupae. larvae and tunnels is impossible and it takes too long to wait for the adults to emerge from the pupae. The species can be separated biochemically by gel electrophoresis of proteins. using one example from each stage (Menken & Ulenberg. 1986). The insects have to be prepared so that the proteins used in the analysis are not modified in any way. One disadvantage of analyzing only one example is that it is impossible to tell if it is a mixed population made up of different species. This was particularly true in tomato crops. This method has also only been developed for four *Liriomyza* species. *L. bryoniae*. *L. huidobrensis*. *L. sativae* and *L. trifiolii*. An additional problem in identification is the presence of parasites in some samples.

Where possible. identification is based on both methods. morphology and gel-electrophoresis. The characteristics of the tunnels. colour of larvae and pupae. as well as the method of pupation were critically observed in order to determine more about the life cycle of *L. huidobrensis* in the Netherlands.

RESULTS

Material was received from different sources for identification. Information was demanded from regional offices of the Plant Protection Service. the Agricultural Information Service. private sources and research institutes. The

spread of *L. huidobrensis* in the Netherlands was not limited to the Westland region. By the use of the measures, which have been taken, large areas of the Netherlands have remained free from attack.

Lyriomyza huidobrensis was found in the following crops in 1989 en 1990:

Campanulaceae
*Trachelium**

Caryophyllaceae
Dianthus cv. Gypsy
D. barbatus
D. caryophyllus
Gypsophila sp.
*Saponaria sp.**
Stellaria sp.

Chenopodiaceae
*Spinacia oleracea** (spinach)
*Beta vulgaris** (sugarbeet)

Compositae
Carduus (thistle)
Cirsium arvense (creeping thistle)
Cichorium endivia (endive)
Aster sp. (aster)
Carthamus sp.
Chrysanthemum sp.
Chrys. frutescens
Gerbera sp.
Petasites hybridus (greater coltsfoot)
Senecio vulgaris (groundsel)
Matricaria sp.
Lactuca sativa (lettuce, iceberg lettuce)

Cruciferae
Brassica oleracea (chinese cabbage)
Raphanus sativus (radish) (white radish
*Matthiola incana** (hoary stock)*

Curcubitaceae
Cumcumis sativus (gherkin) (cucumber)
*Curcubita pepo** (courgette)
*Cucumis melo** (melon)

Gentianaceae
Exacum sp.
Lisianthum sp.

Labiatae
Glechoma hederacea ground-ivy)

Papilionaceae
Phaseolus vulgaris (french bean) (pole bean)
Vicia faba

Primulaceae
Primula sp.

Scropularaceae
Anthirrhinum sp.

Solanaceae
Capsicum annuum (pepper)
Solanum lycopersicum (cherry)tomato
Solanum nigrum (black nightshade)
*Solanum tuberosum** (potato)
Solanum melongena (egg plant)

Umbellifera
Daucus carota (bunching carrot)
Apium graveolens (celery) (celeriac)
Petroselinum crispum (parsley)

Violaceae
Viola sp.

* observed for the first time 1990

Intensive study of the damage showed that the tunnels, especially in summer, were mainly on the underside of the leaves. They were often alongside or in the leaf veins, but this differed between crops. The pupae are yellow brown and usually darker than those of *L. bryoniae*. In about October the pupae become darker and some become virtually black. However, these do not develop rapidly as in the summer and are possibly diapause pupae, for overwintering, which emerge as flies in the spring. It is also noticeable that the tunnels in many crops are more prevalent on the upper leaf surfaces in the winter months. The early attack of *L. huidobrensis* in the spring of 1990 suggested that they over wintered as pupae outside. This leaf miner was observed flying outside above the crop when the sun shone, even on fairly cold days in the spring (beginning April) and autumn (end October) and is clearly less sensitive to cold than *L. trifolii*. The inventory also gave us a better insight into the scale of leaf miner (often not damaging) occurrence in crops.

Control measures and their effects

Joint research carried out by the Plant Protection Service and research institutes has led to the following measures and advice.

Highest priority should be given to the starting material, the plant growers being the first in the chain from grower to consumer. From October 1st, 1989 all growers that produce young plant material had to be virtually free from the flies and any symptoms (tunnels and punctures). Inspection is carried out once or twice a month. Growers who grow plants to maturity are required that they do not cause problems for neighbouring growers.

Compounds such as pyrazophos and triazophos, which work well against *L. trifolii* appear ineffective against *L. huidobrensis*. However, oxamyl and abamectine are effective. For floriculture under glass, complete chemical control is possible by using these chemicals against the larvae. Additional measures against the flies are fumigation with dichlorophos or deltamethrin. Biological control is an effective alternative method for vegetable crops grown under glass. Tests carried out with lettuce by the Glasshouse Crops Research Station in Naaldwijk have given good results with *Dacnusa sibirica*. The parasitic wasps *Diglyphus isaea* and *Dacnusa sibirica* have also been used effectively on fruitvegetables. In a few cases where a very large population was present and cultivation was on Rockwool, oxamyl was dripped onto the growth medium. Leaf crops, such as lettuce grown under glass, are grown in soil treated with oxamyl granules. An additional method of control is the use of synthetic pyrethroids. In between crops eradication can be achieved by steaming the soil or fumigation against any remaining flies. It is also very effective to leave the greenhouse windows closed after the harvest in the summer. This results in temperatures above 60° C. The Plant Protection Service showed that gauze with a pore size of 0.8 mm kept the flies out. Some growers with young plant material use this gauze although only on a limited scale.

DISCUSSION

After the beautiful summer of 1989 there was a mild winter, followed by a warm spring. Therefore, many problems were expected in 1990. In fact these were not as bad as expected.

By visits and giving extensive information the Plant Protection Service made growers aware of the threat of *L. huidobrensis* and information was given as to which chemical compound or other measure was appropriate if there was an attack. Biological control by parasites proved especially a good alternative for crops grown under glass. As well as in glasshouse crops, tunnels were also found in crops such as gypsophila, lettuce, iceberg lettuce and celeriac grown outside. Here many spontaneous cases of parasites appeared. Growers who either export or supply young plants or cuttings obviously require an 0-tolerance for these leaf miners.

The Research Station for Floriculture, Alsmeer; Research Station for Arable Farming and Field Production of Vegetables, Lelystad and the Plant Glasshouse Crop Research Station, Naaldwijk are continuing further research on the use of insect-proof gauze and chemical and biological control measures. It can be assumed that the situation is now under control and that clean plant material can be exported.

L. huidobrensis has been found in greenhouses and sometimes in the open in other European countries and there is an eradication campaign in England. It has been eradicated from a number of pot plant businesses in Denmark, where the chrysanthemum cuttings originated from Costa Rica. It is also possible that *L. huidobrensis* may be present in other European countries, where at the moment there is no means of identifying this insect.

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THE ENTOMOFAUNA OF CONES OF *LARIX DECIDUA* AND *L. KAEMPFERI* IN THE THE NETHERLANDS

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Keywords:
Cone and seed insects, *Larix decidua, L. kaempferi.*

Summary

Megastigmus pictus (Förster) (Hymenoptera: Torymidae) and *Eurytoma laricis* Yano (Hymenoptera: Eurytomidae) caused a loss of 52.6% of the filled seeds harvested in 1986 in the larch hybrid seed orchard at Vaals. In 1988, 47.0% of the seeds of *Larix decidua* Mill. in the same seed orchard were externally damaged by insects; seed chalcids destroyed 69.2% of the remaining filled seeds. A preliminary overview is given of the species new to the Dutch fauna which were reared in 1986, 1989 and 1990 from cones of *L.decidua* and of *Larix kaempferi* (Lamb.) Carr. Two new parasitoids, *Dendrocerus chloropidarum* (Ceraphronoidea: Megaspilidae) and *Rhoptromeris strobigena* (Cynipoidea: Eucoilidae) were reared from chloropid puparia present in the cones.

INTRODUCTION

Larix eurolepis Henry is a hybrid of the European larch, *Larix decidua* Mill. and the Japanese larch, *Larix kaempferi* (Lamb.) Carr. The seed is much in demand because of the hybrid's fast growth and its resistance against the fungus *Lachnellula willkommii* (Hartig) Dennis (Gremmen, 1982). For the production of the hybrid seed, a seed orchard of 2 ha containing clones of both species was established in 1969 at Vaals. Cones are collected from the only *L.decidua* clone. Harvests of hybrid seed have been poor (0.2-4.0 kg) except in 1975 (8.3 kg) and 1977 (38.0 kg). Although the larch trees usually produce good cone crops every 2 or 3 years, a great part of the seed is often empty due to insufficient pollinization of the female flowers on the European larch. In addition, cone and seed insects attacking filled seeds contribute to the reduction of the seed crop. Since 1986, insects attacking cones and seed of *L.decidua* and *L.kaempferi* have been collected and analyses were made to estimate the losses that could be attributed to these insects.

MATERIAL AND METHODS

In 1986, 1989 and 1990, insects attacking cones and seed of *L.decidua* and *L.kaempferi* were reared from cones collected
in the seed orchard at Vaals and in forest stands of these tree species at Wageningen, Bennekom and Grollo. Larvae of Lepidoptera were removed and reared on an artificial diet described by Moraal (1989). Pupae were kept under laboratory conditions until 15 October when they were placed at -2°C until 1 March of the following year. Emerged insects were identified or verified by taxonomists specialized in the families concerned. Two studies were conducted in which seed losses due to insect attack were quantified.

1. Losses caused by seed chalcids. In March 1986, losses were determined in 10 lots of 100 randomly selected seeds of the total seed harvest of *L.decidua* at Vaals. In June, after most insects had emerged, the number of seeds that was empty, filled or still inhabited by insects was determined in a cutting test.

2. Damage caused by cone insects and seed chalcids. In March 1989, an analysis was made of the losses that could be attributed to all insects damaging seeds of *L.decidua*; 40 cones were selected randomly from 350 cones harvested on 2 March in the same seed orchard. All scales were removed from the cones and the number of seeds that had been damaged externally by insects was counted. In June, after most of the insects had emerged, the number of externally undamaged seeds that was empty, filled or still inhabited by insects was determined in a cutting test.

RESULTS

1. Losses caused by seed chalcids. *Megastigmus pictus* Förster (Torymidae) and *Eurytoma laricis* Yano (Eurytomidae) were reared from the seeds. The seed chalcids accounted for a loss of 52.6% of the filled seeds in 1986 (Tab. 1).

Variable	Mean	S.D.	N	Median	Minimum	Maximum
Filled seeds	10.9	1.45	10	11.0	9.0	13.0
Empty seeds	77.0	3.06	10	77.5	71.0	81.0
Seed chalcids	12.1	2.13	10	11.5	10.0	16.0
Seed loss (%)	52.6	0.04	10	52.5	45.5	60.0

Table 1. Statistics of seed losses due to chalcids in 1986.

2. Damage caused by cone insects and seed chalcids (Tab. 2). In 1988, 47.0% of all seeds were externally damaged by insects (Table 2). The species responsable for this type of damage are mainly Lepidoptera (*Cydia illutana, Dioryctria abietella*) and Diptera (*Strobilomyiia* spp., *Resseliella*

skuhravyorum). Of the seeds that appeared undamaged, the majority (85.1%) was empty, while 69.2% of the filled seeds were attacked by the seed chalcids. Per cone, only 2 filled seeds could have been harvested; consequently, the cone harvest was cancelled. The analysis clearly demonstrated that the seed orchard is affected by two major problems, one concerning pollination and the other concerning insects.

Variable	Mean	S.D.	N	Median	Minimum	Maximum
Seeds ext. damaged	40.6	21.6	40	43.0	0.0	80.0
Seeds not ext. damaged	45.7	18.8	40	43.5	10.0	82.0
Total number of seeds	86.4	9.8	40	87.5	63.0	112.0
Empty seeds	38.9	15.8	40	35.0	10.0	77.0
Filled seeds	2.0	3.3	40	1.0	0.0	17.0
Seeds with chalcids	4.6	4.4	40	3.5	0.0	23.0

Table 2. Statistics of seed losses per cone due to cone insects and seed chalcids in 1988.

A preliminary list of the insect species that were reared from the cones and which are new (*) or most probably new (**) to the Dutch fauna is given below. Unless otherwise indicated, the number of specimens exceeded 10 ♂♂ and 10 ♀♀.

Lepidoptera
Species: **Cydia illutana* H.-S. (Tortricidae)
Identification: P.Grijpma, K.J Huisman, J.C.Koster
Coll.: June, July 1989. Em.: May 1990 (lab) 7♂♂,5♀♀.
Locality: Vaals, Wageningen, Grollo
Host: *L.decidua, L.kaempferi*
Comments: Hibernates in the larval stage. Reared on diet from 3d instar larvae to adult.
References: Danilevski & Kuznetsov, 1968;

Diptera
Species: ***Strobilomyiia melania* Ackl. (Anthomyiidae)
Identification: A.Roques
Coll.: June 1989. Em.: March 1990 (lab) 2♂♂,9♀♀.
Host: *L.decidua, L.kaempferi*
Localities: Vaals, Grollo
References: Roques & al. 1983, 1984

Species: ***Strobilomyiia infrequens* Ackl. (Anthomyiidae)
Identification: A.Roques
Coll. June 1989. Em.: March 1990 (lab) 3♂♂,2♀♀
Host: *L.kaempferi*
Localities: Grollo
References: Roques & al., 1984; Roques & v. Hirscheydt, 1990

Species: **Resseliella skuhravyorum* Skrz. (Cecidomyiidae)
Identification: M.Skrzypczynska, W.Nijveldt
Coll.: July 1989. Em.: April 1990 (lab)
Host: *L.decidua*
Locality: Vaals

References: Skrzypczynska, 1975a

Species: *Hapleginella* sp.; *Gaurax* sp. (Chloropidae)
Identification: H. Andersson
Coll.: Jan. 1989. Em. May 1989.
Host: *L.decidua*
Locality: Vaals
References: Roques, 1983; Gaidene & Nartshuk, 1963

Hymenoptera
Species: **Dendrocerus chloropidarum* (Megaspilidae)
Identification: P. Dessart
Coll.: March 1989. Em. March and May 1989 (lab). 2♂♂.
Host: *Hapleginella* sp. and/or *Gaurax* sp. (Chloropidae)
Locality: Bennekom, Vaals
Reference: Dessart, 1990

Species: **Rhoptromeris strobigena* (Eucoilidae)
Identification: G. Nordlander
Coll.: Jan.-March 1889. Em. Feb.-May 1989.
Host: *Hapleginella* sp. and *Gaurax* sp. (Chloropidae)
Locality: Vaals, Wageningen, Grollo
Reference: Nordlander & Grijpma, 1991

Species: **Megastigmus pictus* (Förster) (Torymidae)
Identification: W.J. Gijswijt
Coll.: March 1986, 1989. Em.: April 1986, May 1989 (lab)
Host: *L.decidua, L.kaempferi*
Locality: Vaals, Wageningen, Grollo
Comments: Lanz (1942) already presumed that this chalcid attacked seeds of *L.decidua* since 1927 in The Netherlands.
References: Roques, 1983; Schwenke, 1982

Species: **Eurytoma laricis* Yano (Eurytomidae)
Identification: Z. Bouček
Coll.: March 1986, 1989. Em. April 1986, May 1989 (lab)
Locality: Vaals, Wageningen, Grollo
Host: *L.decidua, L.kaempferi*
Comments: *Eurytoma boučeki* Skrz. from seed of *L.polonica* and *L.decidua*, is possibly a synonym of *E.laricis* (Z.Bouček).
References: Schwenke, 1982; Skrzypczynska, 1975b, 1975c,

Species: **Phaenocarpa seitneri* Fahringer (Braconidae)
Identification: C. van Achterberg
Coll.: June 1989. Em.: April 1990 (lab) 1♂
Hosts: *Strobilomyia* spp. (Dipt., Anthomyiidae).
Locality: Grollo
Comments: *S.infrequens* Ackl. and *S.melania* Ackl. were also reared from the cones of *L.kaempferi*.
References: Seitner, 1929; Van Achterberg & Roques, 1987

Species: ***Aphanogmus strobilorum* Bakke (Calliceratidae)
Identification: P. Dessart
Coll.: August 1989. Em. October 1989 (lab) 3♂♂,3♀♀.
Host: *Asynapta strobi* (Kieffer) (Dipt., Cecidomyiidae)

Locality: Vaals
Comments: Bakke (1955) reared this parasitoid from *A.strobi* present in *Picea abies* cones.
References: Skrzypczynski, 1977; Nijveldt, 1981

Species: **Anogmus laricis* Bouček (Pteromalidae)
Identification: Y.Jongema
Coll.: March 1989. Em. May 1989 (lab)
Host: presumably *Asynapta strobi*
Locality: Vaals
References: Kristek & al., 1976; Skrzypczynska, 1978

Species: **Eupelmus pullus* Ruschka (Eupelmidae)
Identification: Z. Bouček
Coll.: March 1986, Aug.1989 Em. May 1986, April 1990 (lab).
Host: *Resseliella skuhravyorum* Skrz. (Dipt., Cecidomyiidae)
Locality: Vaals
References: Skrzypczynska, 1978

Species: **Pediobius deplanatus* Bouček (Eulophidae)
Identification: Y.Jongema
Coll.: March 1989. Em. May 1989 (lab).
Host: presumably *Hapleginella* sp. and/or *Gaurax* sp. (Dipt., Chloropidae)
Locality: Vaals
Comments: Reared from chloropid puparia in *L.decidua* cones

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AIMS OF THE ANNUAL SURVEY OF INSECT INFESTATIONS ON TREES AND SHRUBS IN FORESTS, ROADSIDE PLANTINGS AND URBAN PLANTINGS IN THE NETHERLANDS

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Keywords:
Survey, insect infestations, trees and shrubs.

SUMMARY

In The Netherlands, insect infestations on trees and shrubs have been surveyed annually since 1946. About 300 voluntary observers throughout the country supply most of the data, which are then used to study the relationships between insect pests and biotic and abiotic factors. Reviews of the occurrence of insect pests are published every year in professional journals, so that the latest information and knowledge is disseminated to the managers of forests and urban plantings.

HOW THE SURVEY IS ORGANIZED

Insect infestations on trees and, to a lesser extent on shrubs, have been surveyed annually in The Netherlands since 1946 (Luitjes & Voûte, 1956). Currently, almost 300 observers, most of whom are professionally interested in the health of their trees, are involved in the survey. About 60% of the observers are forest managers. The remainder (tree surgeons, local authority parks and gardens staff, etc.) collect data on insect pests on trees and shrubs in urban plantings.

To reduce the danger of misidentification, the observers are trained by a team of provincial staff who arrange excursions and lectures annually. Furthermore they are provided with handbooks, identification keys, publications and other literature. The observers are urged to send in specimens of insects or a sample

of infested material if they are doubtful about identification. They are given forms on which to record the following data: insect species, tree species and age, location and national grid reference (5x5 km), the degree of infestation (light, moderate or heavy) and an estimate of the area infested. This method of collecting and processing survey data differs from that used by the European Invertebrate Survey (EIS)(van Tol, 1979). A database management and query system (ORACLE) was introduced in 1978. It was recently improved, and a database containing climatic factors was incorporated (Seigers, 1989).

THE AIMS OF THE SURVEY

The annual survey involves an ongoing overview to detect, monitor and assess the occurrence, frequency and significance of insect pests (Luitjes & Voûte, 1956). The history of an infestation, specially the number of consecutive years of defoliation, can be used to draw conclusions about the probable vitality or mortality of the trees or stands. Furthermore, the relationships between the occurrence and severity of infestations, climatic factors (in the future possibly even climatic change) and site factors can be analysed (Power & Williams, 1987).
One of the ways the database has been used is to study the frequency and significance of forest insect species in specific areas such as the new polders in The Netherlands (Grijpma & Glastra, 1983). In combination with other factors such as acid deposition, adverse weather and diseases, insect pests may also play a role in the frequently observed decline in tree vitality. This is why it is important to study these pests. It is known that climatic factors are important in relation to some plagues of insect pests. Data from the Dutch insect survey have already been used in preliminary mathematical models to describe how the occurrence of infestations of *Operophtera brumata* and *Tortrix viridana* on *Quercus* is related to climatic conditions. The following factors are important in these models: * Synchronicity between hatching of the eggs and bud burst.
* The severity of the winter. * The population density of the insects in the previous years (Andriesse, 1990; Leffef, 1988).

In the future the use of Geographical Information Systems (GIS) may result in a further stratification of infestation areas and may provide information on trees appropriate for planting along streets and in other public areas, taking variation in tree shape and size into account. GIS techniques can reveal relations between areal features (such as adaptation to soils and climate) and susceptibility to disease and insect attack (Power & Williams, 1987). The forest monitoring network in France is an example of an advanced survey organization. The French use a computerized system (Télétel) for transmitting and receiving information. They input data on diseases, pests and other biotic and abiotic factors directly in a database, together with other relevant parameters about vegetation and soil etc. (Département de la santé des forêts, 1990).

The Dutch observers also monitor new pests. During the last twenty years coccoid scales of exotic origin (*Pulvinaria regalis* and *Eupulvinaria hydrangeae*) have become pests on trees in cities in Western Europe (Merlin et al., 1988). They have spread northwards and have recently become pests in The Netherlands too. The annual survey of tree pests allows the spread of the insects to be traced. At present *E. hydrangeae* is observed mostly in the cities in the south of The Netherlands. *P. regalis* has so far only been reported from cities (Amsterdam and Utrecht) in the centre of the country (Moraal, 1988; 1989).

When the survey signals an outbreak of a new or rare pest, a provisional ecological study can be done, because large numbers of the insects and their parasites can easily be collected. The data can also be used to help decide which pests should be studied in new research projects.

Reviews of the occurrence of insect pests in The Netherlands are published annually in professional journals (Moraal, 1990a, 1990b). In this way, the latest information and knowledge is disseminated among managers of forests and urban plantings.

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CHEMOSENSORY PERCEPTION OF LEAF SURFACE CHEMICALS BY OVIPOSITING *PIERIS BRASSICAE* L. BUTTERFLIES

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Keywords:
Chemoreception, phytochemistry, kairomones, oviposition, *Pieris brassicae*.

Summary

Methanolic extracts of the surface layer of intact cabbage leaves induced a significant preference over solvent controls when applied on artificial leaves in dual choice oviposition tests with large white butterflies, *Pieris brassicae* L. Extracts prepared using either apolar solvents or water were inactive. After a molecular size-based separation of compounds in the methanolic extract into 20 fractions, activity was present only in a subset of these. Recordings of the electrophysiological activity in tarsal chemosensilla in response to these fractions revealed that only the fractions belonging to the behaviourally active subset elicited responses. At lower doses of the methanolic extract, responses originated mainly from one of the four chemosensory neurones present in the sensilla, indicating a straightforward reflection of taste perception on the behavioural level.

INTRODUCTION

Contact chemoreception is known to play a crucial role in host plant selection behaviour of plant feeding insects (Schoonhoven, 1981). The majority of plant chemicals that either deter or stimulate insect feeding or oviposition by gustatory action have been found after chemical extraction of the whole leaf, which predominantly consists of interior leaf components. In recent years, research interest has gradually been focussed on the undamaged plant surface as the initial contact substrate (Städler, 1986; Chapman & Bernays, 1989). Insects are equiped with numerous gustatory sensilla that provide them with detailed information about the chemical composition of the contacted substrate (Frazier, 1986). The cabbage white butterfly species *Pieris brassicae* L. and *P. rapae* L. (Lepidoptera: Pieridae) accept for oviposition only cruciferous plants and some members of other plant families that all contain specific secondary plant compounds called glucosinolates. David & Gardiner (1962) have shown that *P. brassicae* females oviposited on green paper which had been treated with the commonly occurring glucosinolate sinigrin. Ma & Schoonhoven (1973) demonstrated that young *Vicia faba* L. plants, normally refused as oviposition substrate, were accepted after systemic uptake of either one of three glucosinolates. Ablation studies showed that tarsal chemoreceptors are necessary for normal oviposition behaviour. Cabbage butterflies perform a sequence of behavioural elements that involve contact testing with the tarsi ('drumming') before actual oviposition takes place. Visual inspection of the site where the female has drummed the leaf shows no signs of penetration of the leaf cuticle suggesting that

contact cues are present on the surface. This study was undertaken to investigate if surface extracts from undamaged leaves were able to induce oviposition. Crude extracts were prepared using solvents of widely different polarity and subsequently fractionated in a first attempt to purify the stimulating surface chemicals. Fractions were tested both on the behavioural and the sensory level to see if correlations existed.

MATERIALS AND METHODS

Female *Pieris brassicae* (L.) were obtained from a laboratory colony maintained for several generations on *Brassica oleracea* L. Rearing conditions were similar to those described by David and Gardiner (1962). Oviposition preferences were tested in cages measuring 80 x 50 x 100 cm high. The egg distribution occurring in a dual choice situation offered in one such cage was considered a replicate. The cages were kept in a conditioned greenhouse, with temperatures fluctuating between 21 °C and 25 °C. In addition to normal daylight, each cage was illuminated from 6.00 to 22.00 hr by a 400 Watt mercury vapour lamp hanging 30 cm above the glass roof of the cage. In each cage the ratio males:females was 2:1. The number of females per compartiment was 6 - 10 (Table 1). Leaves of 6-8 week old *Brassica oleracea* var. gemmifera *cv.* Titurel plants were excised, weighed and dipped during 5 s in one of the following solvents or sequences of solvents: A. hexane-chloroform-methanol; B. hexane-dichloromethane; C. dichloromethane-methanol; D. methanol; E. water. Dipping was performed at 25 °C, except the water-dip which was done at a water temperature of 80 °C. After dipping the petioles were excised from the lamina and weighed separately and the difference with the total leaf weight gave the number of gram leaf equivalents (gle) dipped in the solvents. The crude methanoldip (80 gle) was brought onto a Sephadex LH20 column and separated into a first fraction of 40 ml and 19 fractions of 10 ml each (elution solvent methanol). This column is chemically inert, is stabile with the solvents used and has the property to separate compounds in the molecular weight range of MW 100 - 2000 on the basis of their size. The chemical stimuli studied in both behavioural and electrophysiological tests were (1) sinigrin (5 mM); (2) hexane-extract; (3) dichloromethane-extract; (4) methanol-extract; (5) waterextract and (6) the 20 Sephadexsubfractions of the crude methanolextract mentioned above. In behavioural tests, the stimuli or solvent controls were sprayed evenly on the upper side of artificial leaves, being green cardboard circles (diameter 9.6 cm). The dose applied with the crude methanol extract was 2.5 gle/artificial leaf, for the Sephadex-fractions a dose of 1.5 gle/artificial leaf was used. The number of batches and total number of eggs laid on control and treated artificial leaves during a 7-8 h period were counted for each replicate cage. For electrophysiological tests, the tip-recording method was employed. Forelegs of 3 days old females were used that had not yet been into contact with cabbage plants. The legs were amputated at the proximal part of the femur and a silver wire electrode was inserted in the femur. This electrode was connected to a biological preamplifier. Details of recording method and equipment have been described elsewhere (Van Loon, 1990). The electric events were recorded on line using a Hewlett Packard Vectra ES12 personal computer equiped with an analog-digital converter (DAS 50, Metrabyte Co.). Recorded signals were analysed by means of the software package SAPID (Mitchell *et al.*, 1990). A glass capillary electrode contained the stimulating or the control solution and was applied to the distal tip of the sensillum. Control solutions were KCl (10 mM) or a mixture of KCl (10 mM) and 3% methanol (for the methanolextract and its fractions).

Table 1. Oviposition on artificial substrates by *Pieris brassicae* females in a dual choice situation (control *vs.* treated). Treatment consisted of application of methanolic surface extracts collected by dipping at four different occasions.

dip#	control	treated	P_{exp}	n_{rep}	N_f
1	145	857	0.05	6	6
	0	706	*n.s.*	6	6
2	56	545	0.05	4	10
	671	1173	*n.s.*	4	10
	445	1022	*n.s.*	4	10
3	438	2218	0.05	6	9
	66	258	*n.s.*	6	9
	335	1695	0.05	6	10
	399	930	0.05	5	8
4	196	857	0.05	6	8
	0	191	*n.s.*	6	10
Total	2751	10452	0.003[a]		
%	21	79			

a - *P* value based on the 11 experiments presented; P_{exp} refers to the experiment-wise significance level (Wilcoxon's signed rank test, one sided); *n.s.* - $P>0.05$; n_{rep} is the number of replicate cages during a one day's experiment; n_f is the number of females per cage.

RESULTS

Behaviour - Of the different surface extracts assayed, only the methanolic dip of the dichloro-methane-methanol solvent sequence resulted into an overall significant preference of treated artificial leaves at the dose tested (Table 1). Remarkably, when methanol was used as the only extraction solvent, it apparently lacked sufficient stimulating compounds. After the active methanolic dip had been subjected to separation over the Sephadex column, 18 of the resulting fractions were bioassayed in 6 sets of three pooled fractions at a dosis of 1.5 gle/leaf. None of these sets induced any noticeable preference of the ovipositing females. Likewise, when fractions 1-10 and 11-20 were pooled, neither of both was active. When all 20 fractions were recombined, however, activity was restored to extent of the parent methanolic extract. Based on electrophysiological results (see below), it turned out that only pooling of fractions 6-13 resulted in significant ovipositional preference of females for treated substrates. Combinations of the remaining 12 fractions were inactive. However, when tested against the parent methanolic extract, pooled fractions 6-13 were significantly less preferred, indicating that stimulatory activity had been lost.

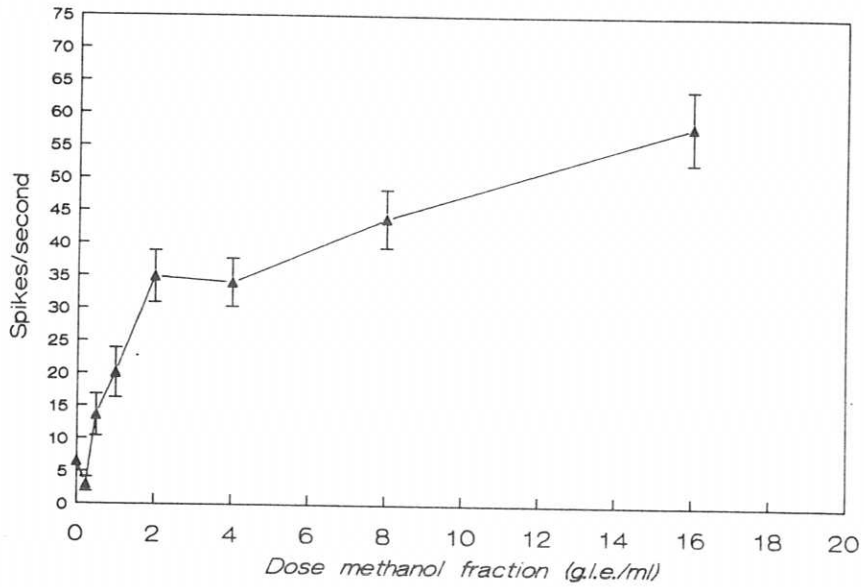

Fig. 1. Dose - response curve for the chemosensory activity (total number of spikes/s) evoked in tarsal chemosensilla of P. brassicae by methanolic surface extracts of cabbage leaves.

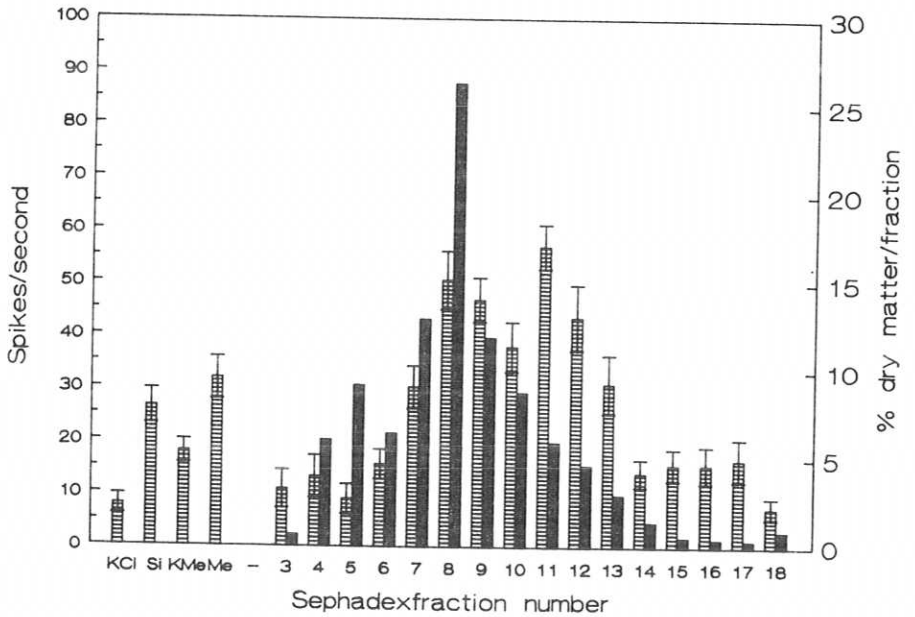

Fig. 2. Intensity of chemosensory activity (total number of spikes/s) elicited in tarsal chemosensilla of P.brassicae in response to different stimuli. KCl - 10 mM potassium-chloride; Si - sinigrin 5 mM; KMe -mixture of 10 mM KCl and 3% methanol; Me - crude methanolic surface extract of cabbage leaves; Sephadexfractions (3 - 18) - see text.

Electrophysiology - The methanolextract that exerted behavioural activity, elicited a significant dose-dependent response from tarsal B-type chemosensory hairs (Fig. 1). While a single cell was predominantly active at lower doses, at higher doses responses were more frequently multicellular. Response threshold was ca. 0.5 gle/ml. Pronounced differences in electrophysiologial activity were observed between the respective Sephadex fractions (Fig. 2). Active fractions differed considerably in dry matter content (*e.g.* fractions 8 and 12, Fig. 2). Electrophysiological activity coincided with behavioural activity in fractions 6-13.

DISCUSSION

This study has demonstrated that in the surface of cabbage leaves compounds are present that stimulate oviposition behaviour of *P. brassicae* females. These compounds must be of a relatively polar nature as they preferentially dissolve in methanol as opposed to apolar extraction solvents like hexane. However, the prior removal of apolar material is necessary to extract the active compounds in methanol. The presence of polar compounds in the apolar environment of the waxy leaf surface has been documented for several plant species as has the perception of these compounds by phytophagous insects (Städler, 1986). The fractionation of the crude methanol extract by means of the Sephadex column resulted into a slight purification of active compounds, although both behavioural and electrophysiological active fractions also had the highest dry matter contents (Fig. 1). A particular combination of fractions was necessary for stimulation of oviposition behaviour. This suggests that several compounds together are responsible for the observed effects. If a further reduction in number of fractions belonging to the pooled set 6-13 can be reached without loss of activity, should be revealed by new test series.

The number of replicates in the dual choice oviposition assays was low (Table 1). This resulted in a lack of experiment-wise statistical significance in 5 out of 11 cases. When in one or two of the replicates of a single-day test series no eggs were produced at all (*P. brassicae* females produce on average less than one batch per day over their life-span), the Wilcoxon statistic led to the conclusion that females had not shown a significant preference, although the remaining replicates showed consistent preference for the treated artificial leaves. Another problem associated with the particular substrate arrangement used was what can be called a carry-over effect: when the treatment was highly stimulatory, the control also received eggs. Direct behavioural observations learned that this was caused by disturbance of ovipositing females by landings of conspecifics. Once in the process of oviposition on the treated artificial leaf, disturbance often led to a new landing on the unoccupied control within a few seconds, after which oviposition was continued immediately.

A problem inherent to bio-assaying surface chemicals is the expression of the doses used and relating these to actual concentrations present in the intact leaf surface. The gle/ml-expression of dosis should in fact be replaced by a gle/unit of surface area expression, but the latter is difficult to assess accurately. It is unknown what proportion of active surface chemicals is extracted. It is probable that extraction of active chemicals has not been exhaustive, due to the short dipping time employed. This was done deliberately to prevent the extraction of chemicals present in the leaf interior. Consequently, the doses used in the bioassays may have represented an considerable underestimation of the natural dose encountered by the butterflies on a normal leaf

surface. Presence of interior leaf chemicals cannot be entirely ruled out. It is possible that during dipping a minor leakage of constituents from the intercellular space has occurred through the cuticular stomata. Absorbance measurements did not yield any detectable amount of chlorofyll in the extracts. Electrophysiological testing of the different Sephadex fractions on tarsal B-hairs provided a clear indication which fractions contained gustatory stimulants. Furthermore, behavioural stimulation was found to reside in the same fractions, which points to a rather direct reflection of chemosensory perception on the behavioural level. Responses to the lower doses of the crude methanol extract were mainly unicellular, although differences between sensilla on a single tarsus were found. Only at higher doses one or two other cells also became active. The dose-response curve is based upon total spike numbers and shows a bimodal shape. After an initial saturated part (between 2 and 4 gle/ml), the response levels rise again with increasing doses and do not reach saturation at 16 gle/ml. One possible explanation for this phenomenon is the following. At higher doses of the crude extract, which consists of a mixture of compounds, the concentration of relatively uneffective compounds may rise above threshold values for stimulation of either the same cells responsive to the lower doses or one or two of the other cells of the four innervating the tarsal chemosensilla.

The results reported by David and Gardiner (1962) and Ma & Schoonhoven (1973) suggest that glucosinolates are interpreted as oviposition stimulants and thus act as kairomones to these butterflies. However, their experimental setups do not allow the conclusion that these compounds are indeed the oviposition stimulants that the butterflies positively respond to when they accept any particular cruciferous plant. The present study is a first attempt to extract and purify stimulatory chemicals from the leaf surface of a host plant. Further isolation, purification and identification are necessary future steps to establish the nature of the kairomones involved.

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VENOMS OF THE HYMENOPTERA - A LEAD TO NEW PESTICIDES?

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Keywords:
Wasp venoms, ant venoms, neurotoxins, natural insecticides.

Summary

Many hymenopteran insects incapacitate their prey by means of a neurotoxin. Nicotinic synaptic transmission in the insect CNS is presynaptically and irreversibly blocked by kinins, which probably are non-competitive choline-uptake inhibitors. Glutamatergic neuromuscular transmission in insects is reversibly blocked by philanthotoxins which are cation channel blockers. Poneratoxin, a 25 amino acid residue polypeptide is the first described hymenopteran neurotoxin affecting excitability of nerve and muscle fibres by changing the kinetics of the voltage-dependent sodium channel.

INTRODUCTION

Several groups of hymenopteran insects, like wasps and ants, sting their prey to a paralysis or only to a behavioural inactivation, in order to offer the prey to the own offspring as an incapacitated, but living source of food. These wasps and ants have developed venoms as natural insecticides.

The ideas of the 1980's included the possibility of using natural neurotoxins from arthropods as leads to new pesticides (Piek, 1982a, 1987; Piek et al., 1988; Hildebrand, 1988). For primary synthesis products like polypeptides, this might be effected by means of genetic engineering and it might also be possible to design a mimic pharmacophore (loop, etc.) of a peptide (Milner-White, 1989).

From venoms of Hymenoptera, selected by nature to incapacitate insects and spiders, two types of neurotoxins have been isolated. The

first group consists of low molecular weight neurotoxins containing a polyamine moiety. From the venom of the sphecid wasp Philanthus triangulum a philanthotoxin was isolated and chemically characterized (Fig. 1, Piek et al., 1988). An identical toxin was isolated from the venom of a subspecies P.t.abdelcader from Egypt (Eldefrawi et al., 1988). Based on the structure of the natural philanthotoxin, now called philanthotoxin-4.3.3 (PTX-4.3.3), a large number of structural analogues were described (Piek & Hue, 1989; Nakanishi et al., 1990; Bruce et al., 1990; Karst et al., 1990 a, b, 1991; Karst & Piek, 1991).

The second group of hymenopteran neurotoxins consists of polypeptides. An example of such neurotoxins are the wasp kinins, originally found in vespid wasp venoms (Nakajima, 1986) and also in the venom of a scoliid wasp, Megascolia flavifrons (Piek et al., 1983, 1984; Yasuhara et al., 1987). The latter wasp stings larvae of the rhinoceros beetle in all nerve ganglia, causing an irreversible paralysis.

Kinins were also found in ant venoms, together with antagonists of unknown composition. Recently one of the latter compounds was chemically characterized as an 25 amino acid residue polypeptide and was called poneratoxin (Piek et al., 1991 a,b).

THE REVERSIBLE NON-COMPETITIVE BLOCK OF SYNAPTIC TRANSMISSION BY PHILANTHOTOXINS

In the 1960's investigations started in our laboratories on the paralysing venom of the sphecid wasp Philanthus triangulum. The excitatory glutamatergic neuromuscular transmission of insects is antagonized by the venom and by its most active toxin (Fig. 1, philanthotoxin-4.3.3, PTX-4.3.3) through two different effects: by a presynaptic inhibition of the high affinity glutamate uptake (Van Marle et al., 1982, 1983, 1986) and by a block of open ion channels of the glutamate receptor-ionophore complex (Clark et al., 1982; Piek, 1982).

Structure-activity relationship studies (Karst et al., 1991, Karst & Piek, 1991) showed that the activity of these polyamines as channel blockers depends on the presence of the aromatic moiety.

Attempts to change the molecule into a more potent antagonist resulted in an increase in potency, for the different analogues, with

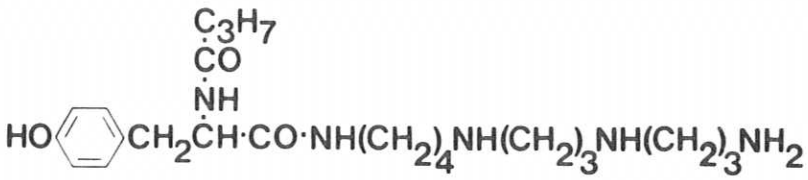

Fig. 1 Delta-philanthotoxin, PTX-4.3.3

factors varying from 10 to 100 (Nakanishi et al., 1990; Bruce et al., 1990; Karst et al., 1991, Karst & Piek, 1991). The pre- and post-synaptic inhibiting properties of the natural PTX-4.3.3 were dissociated in two different analogues (Karst et al., 1990b).

NON-COMPETITIVE INHIBITION OF CHOLINE-UPTAKE BY KININS

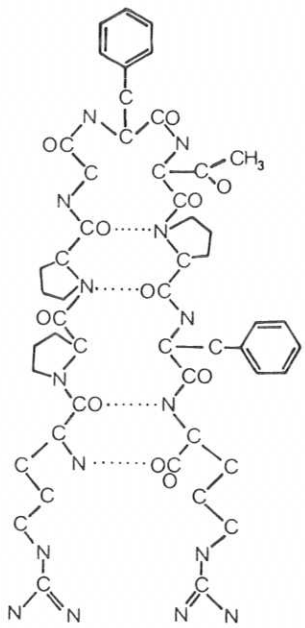

Fig.2 - Threonine-6-bradykinin

The most active low mol.wgt fraction of the venom of Europe's largest wasp, Megascolia flavifrons contains threonine-6-bradykinin (Thr^6BK, Fig. 2). Intra-ganglionic perfusion of the sixth abdominal ganglion of the cockroach, Periplaneta americana, for a short period of time, followed by a long period of wash, caused a slow and progressive decrease in synaptic transmission from the cercal nerves to an identified giant interneuron. Since, the kinin did not affect iontophoretically applied acetylcholine potentials, the activation-induced

block proved to be presynaptic (Hue & Piek, 1988, 1989). In the micromolar range Thr^6BK is at least twice as potent as hemicholinium-3, a competitive inhibitor of the choline uptake (Schueler, 1960). Thr^6BK however, causes a non-competitive inhibition of the choline uptake (Prof.Heinz Breer, Univ.of Stuttgart, personal comm.).

PONERATOXIN, THE FIRST HYMENOPTERAN TOXIN AFFECTING VOLTAGE DEPENDENT SODIUM CHANNELS

Several myrmicine and ponerine ant venoms contain kinins as well as different neuroactive antagonists (Piek et al., 1989).

```
Phe-Leu-Pro-Leu-Leu
                   |
Leu-Ser-Gly-Leu-Ile
|
Leu-Met-Thr-Pro-Pro
                   |
Ile-Ala-Gln-Ile-Val
|
His-Asp-Ala-Gln-Arg
                   |
                  NH2
```

Fig. 3 - Poneratoxin

One of these venoms, from Paraponera clavata, causes a strong contraction of mammalian smooth muscles and fibrillation of skeletal muscles (Schmidt et al., 1984; Piek et al., 1991 a, b). At concentrations from 10^{-8}M - 10^{-6}M the most active toxin, called poneratoxin (PoTX; Fig. 3) affects the voltage-dependent excitability of the isolated cockroach axons as well as of isolated frog and rat skeletal muscle fibres. PoTX prolongs action potentials and induces slow automatic activity due to a slow Na^+-current activation at very negative values of membrane potentials and due to slow deactivation. An extensive description of this action is in preparation.

DISCUSSION

Criticism on the possibilities to use venom toxins as new leads in pesticide science has been, that these compounds have to be injected into the insect body or even into the CNS. However, it becomes possible now to incorporate the genetic codes for the biosynthesis of peptides and proteins in entomophilic viruses. In this way it may be possible to let viruses produce small amounts of toxins in the insect

body. If these toxins block synaptic transmission irreversibly, a pesticide action becomes possible.

For non-primary synthesis products like the polyamine compounds, change in chemical structure seems the only way to obtain potential pesticides.

The majority of insecticides used today are neurotoxins, many of them changing the kinetics of the voltage-dependent sodium channels. Arthropod venoms like wasp and ant venoms also contain antagonists of synaptic transmission processes and may provide leads for new approaches to insecticide development.

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QUANTITATIVE ASPECTS OF PHOTOPERIODIC TIME MEASUREMENT IN THE SPIDER MITE *TETRANYCHUS URTICAE*

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Keywords:
Diapause, photoperiodism, quantitative photoperiod perception, *Tetranychus urticae*.

Summary

Current models for the photoperiodic clock are based on a qualitative principle of nightlength measurement; the clock determines only wether nights are longer or shorter than the critical nightlength. For some insect species it has now been shown that nightlength measurement takes place according to a quantitative principle; the inductive "strength" of a night depends on its absolute length.

Quantitative aspects of the time measurement mechanism of the spider mite *Tetranychus urticae* are investigated, using special experimental techniques.

Introduction

In the temperate zones, diapause is an adaptation of many insect and mite species, which enables them to survive seasons unfavourable for development and reproduction. Besides temperature, photoperiod is the most important factor controlling diapause induction. In general, long days give continuous development, while short days result in the incidence of diapause. Often insects and mites are able to discriminate long days from short days with great accuracy (Saunders, 1982; Veerman, 1985).

Little is known of the physiological mechanism of diapause induction. The mechanism is thought to consist of a receptor part and an effector part. The receptor part includes a photoreceptor, a photoperiodic clock and a photoperiodic "counter". In general, nightlength has been found to be more important than daylength in photoperiodic time measurement. Therefore, the clock seems to discriminate between long and short nights. For induction to take place, insects and mites have to experience

a certain number of long-night cycles; this summation of the effect of a series of photoperiodic cycles has led to the concept of the photoperiodic counter (Saunders, 1982).

Over the past decades, much research has been done on the mechanism of the photoperiodic clock, resulting in various models for time measurement. A common feature of the current photoperiodic clock models is that they are based on a qualitative principle of time measurement (Tyshchenko, 1966; Lees, 1981; Pittendrigh, 1981; Vaz Nunes & Veerman, 1982; Lewis & Saunders, 1987). In qualitative perception of nightlength, the mechanism determines whether nights are longer or shorter than a certain threshold. This threshold separates the domains of two distinct responses, the long day-response and the short day-response. This threshold is called the critical nightlength.

However, for some insect species it has now been shown that nightlength measurement takes place according to a quantitative principle (Zaslavski, 1988). According to this principle, the animals are thought to measure the absolute length of the night. In this case not every long night necessarily has the same inducing strength. According to Zaslavski (1988), quantitative time measurement is common in insects and mites.

There are several possible ways to investigate the nature of photoperiodic time measurement. First, an experimental design may be used in which both the number of light-dark cycles given and the length of the night are varied. In this way the dependence of the number of long-night photoperiodic impulses, needed to induce diapause, on the concrete nightlength can be studied, thus making it possible to discriminate between the two possible ways of nightlength perception (Zaslavski, 1988). A second possibility to investigate the nature of photoperiodic time measurement is an experimental design called a two-step photoperiodic reaction experiment. Sometimes stepwise reactions reveal special features that are masked in experiments carried out in constant photoperiods. In a two-step reaction experiment the photoperiodic conditions experienced during individual development are not stationary but consist of two different photoperiodic regimes (Zaslavski, 1988).

An example of a two-step reaction is the specific photoperiodic reaction of the lacewing *Chrysopa carnea*, occurring when the insects experience a change in photoperiodic conditions (Tauber & Tauber, 1970). Under constant photoperiods the lacewing displays a common long-day reaction; all adults enter diapause at a daylength of 13 hours or less. However, if the animals experience during individual development an increase in photophase from 8 to 12 hours, which is within the short-day range and far from the threshold value, reproductive activity ensues.

In the present work, quantitative aspects of the photoperiodic mechanism of the spider mite *Tetranychus urticae* were studied, using both methods mentioned above.

Material and Methods

In this study the 'Sambucus' strain of *T. urticae* was used. This Dutch strain was collected in Voorne in 1961 and has been reared in the laboratory ever since. The stock culture of the mites is maintained under long-day conditions (LD 17:7) at 25°C on bean plants (*Phaseolus vulgaris*). Criteria for diapause in *T. urticae* were described by Veerman (1977, 1985).

For each photoperiodic treatment about 90 adult females were kept on 3 detached leaf cultures of bean. Within 24 hours the females were removed from the leaves and the eggs, approximately 250 eggs per 3 leaves, were maintained for 4 days at 26°C ± 0.5°C and continuous light in a light and temperature controlled incubator, at which time the eggs were ready to hatch. During postembryonic development, the mites were exposed to various light-dark regimes at 20°C ± 1°C in a number of light-proof wooden cabinets, placed in an environmental room. Each cabinet was equipped with 2 daylight 8-W fluorescent tubes, which were seperated from the working space of the cabinet by a perspex screen. The light-dark cycles required could be generated independently in the cabinets and were controlled by electronic timers. Light intensity at the level of the mites varied from 500 to 700 lux.

In the stepwise photoperiodic reaction experiment mites were exposed to 0 - 18 cycles of LD 8:16. The rest of the postembryonic development took place at LD 12:12, after which the number of diapause and non-diapause females was counted.

In the counter experiment mites were exposed to 0 - 15 cycles of an inductive long night regime, starting at egg-hatch. The mites were maintained in continuous light for the rest of their development, until the percentage diapausing females could be determined. The long-night regimes tested were LD 10:14, LD 6:18 and LD 2:22. When necessary deteriorated bean leaves were replaced by fresh ones.

Results and Discussion

The results of the stepwise reaction experiment are listed in Table 1. In this experiment no two-step reaction could be discovered, since a stepwise change in long-night regime did not affect diapause induction. However, other regimes have to be

tested, before it can be decided whether or not *T. urticae* displays a stepwise photoperiodic reaction.

Table 1.
Diapause induction in T. urticae in response to various sequences of LD 8:16 and LD 12:12. Day of photoperiodic change means the day of transition from LD 8:16 to LD 12:12.

day of photoperiodic change	number of females tested	diapause (%)
0	227	100
1	326	97
2	423	100
3	371	99
4	339	99
5	233	97
6	308	99
7	370	99
8	413	100
9	370	100
10	429	100
11	502	100
12	452	100
13	398	100
14	371	100
15	230	100
16	249	100
17	189	100
18	220	99
19	206	100

Zaslavski (1988) listed stepwise photoperiodic reactions of 61 insect species. Sometimes these reactions are clearly recognizable, for instance in the case of the photoperiodic reaction of the lacewing *Chrysopa carnea* described in the introduction. The stepwise reaction has also been demonstrated at photoperiods very near the critical nightlength (Fomenko & Zaslavski, 1978). However, near the threshold region there often is a considerable individual variation in photoperiodic reaction. Therefore, one should be very cautious in drawing conclusions about the photoperiodic mechanism when near-critical photoperiods are studied.

The results of the counter experiments are shown in Figure 1. Using an excessive number of cycles diapause incidence was found to be nearly 100% under all three photoperiods tested (LD 10:14; LD 6:18 and LD 2:22). But when a smaller number of cycles was given, differences in inductive strength between the long night regimes became clear. The mites needed only 8 cycles of LD 10:14 to reach a diapause incidence of 50%. 11 and 12 cycles, respectively, were needed with the photoperiods LD 6:18 and LD 2:22 for 50% diapause induction. 10 cycles of LD 10:14 were sufficient for a

nearly saturated diapause response (97%), while after 10 cycles of LD 6:18 diapause incidence was found to be 30% and after 10 cycles LD 2:22, only 8%. According to these results different long nightlengths, all far from the critical nightlength, may differ considerably in inductive strength in *T. urticae*.

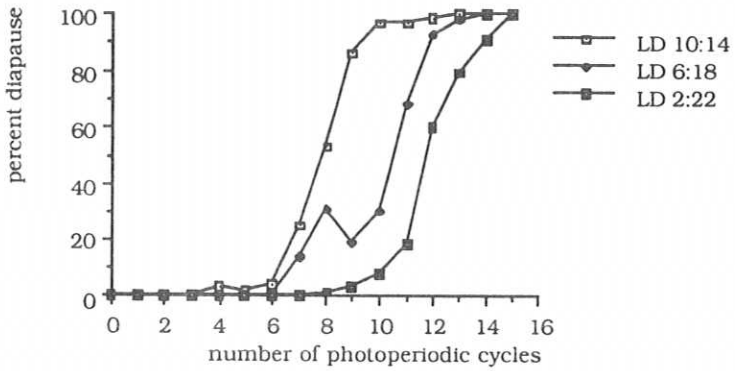

Figure 1.
Diapause incidence in T. urticae in response to a varying number of long night cycles of 3 different long night regimes (LD 10:14, LD 6:18 and LD 2:22).

The results also show that there is a great individual variation in response to numbers of long-night cycles within one photoperiodic treatment. In the Sambucus population some mites needed only 4 LD 10:14 cycles for diapause induction, whereas other mites needed more than 10 cycles. In research on photoperiodism the photoperiodic response of a population is studied; an individual has only two alternatives, either to enter diapause or not. This makes the investigation of the photoperiodic clock mechanism, which is a characteristic of an individual, more complicated. It may be good, therefore, to repeat the counter experiments with an inbred line of the Sambucus strain, in which much less individual variation is expected to be present.

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THE ROLE OF TERPENOID END PRODUCTS IN DEVELOPMENT OF THE APHID *MEGOURA VICIAE* (BUCKTON)

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Keywords:
Farnesenes, monoterpenes, polymorphism, sesquiterpenes.

Summary

Terpenes are involved in morphogenesis of aphids. Polymorphism in *Megoura viciae* is affected by external factors that induce characteristic shifts in monoterpenes and sesquiterpenes. The role of a key enzyme is discussed.

INTRODUCTION

Many aphid species show wing dimorphism in parthenogenetically reproducing females in summer and produce sexual forms in autumn. Although Juvenile Hormone (JH) was originally thought to govern the processes underlying aphid polymorphism (Hardie, 1987), its function is probably limited to holocyclic species where short photoperiod evokes in gynoparae, giving birth to oviparous females and males. Long days activate specific neurosecretory cells in the brain triggering a sufficiently high JH_{III} activity to induce the production of virginoparae. Different environmental factors such as crowding affect the internal regulation of wing dimorphism in these virginoparae.

JH_{III} is one end product of the mevalonate metabolism, other possible end products being ecdysone and farnesenes. Farnesenes can act as semiochemicals: the structure of the alarm pheromone of the aphid *Myzus persicae* (Sulzer), E-ß-farnesene was elucidated by Bowers et al. in 1972. E-ß-farnesene is not the only farnesene produced by aphids. Minor quantities of other isomers, like (Z,E)-α-farnesene and (E,E)-α-farnesene can be produced by the same species.

We found both the ratio and amounts of these isomers to change with the production of different morphs in *M. persicae* (Gut et al., 1987). Growth and development of *Aphis fabae* and *M. persicae* could be strongly inhibited by external application of (E,E)-α-farnesene and wing dimorphism of a few biotypes of the latter aphid could be directed into either the apterous or alate course of development with natural end products of other pathways of the mevalonate metabolism (Van Oosten et al., 1991). These results suggest that mevalonate metabolism in aphids is functionally related with developmental processes involved in polymorphism. In mammals, the synthesis and activity of end products of the

mevalonate metabolism is governed by multi-valent feed-back mechanisms, in which a key enzyme, hydroxymethylglutaryl coenzyme A reductase (HMG-CoA reductase) has an important function in the production of precursors of the end products, such as cholesterol (Goldstein & Brown, 1990). In many aphid species, however, it is difficult to establish the functional significance of farnesene isomers, as they are all sesquiterpenes the synthesis of which is closely related to that of JH_{III}. The aphid *Megoura viciae* produces the monoterpenes nepetalactol and nepetalactone, proven to be sex pheromones of this aphid (Dawson et al., 1987). When farnesenes would be involved in polymorphism of *M. viciae*, the ratio and amount of monoterpenes and sesquiterpenes in photoperiodically conditioned aphids could provide essential information on the role of regulating enzymes.

MATERIALS AND METHODS

- Aphids

A stock culture of parthenogenetic generations of *Megoura viciae* was maintained on bean plants in a climatized glasshouse at 17 ± 2° C, with a 16h photoperiod. Experimental aphids for long-day (LD) and short-day (SD) regimes were transferred during their fourth larval stage to young bean plants of the same cultivar and placed in identical climate cabinets, one set at L:D 16:8h and 18:15° C, the other at L:D 12:12h and 15:12° C. Fig. 1 shows the rearing procedure to synchronize individual development.

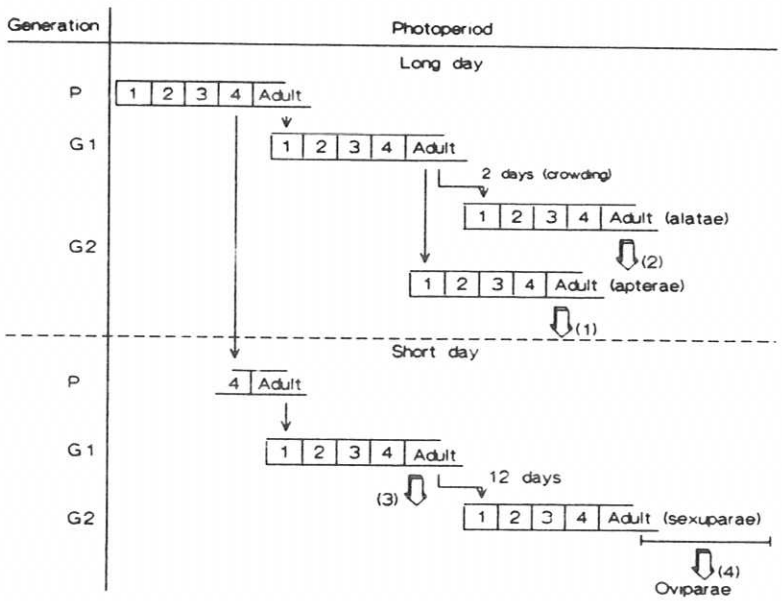

Fig. 1. Rearing procedure to obtain samples of M. viciae *of different morphs. The P generation was produced by alate mothers. The first generation born under experimental conditions is G_1. Open arrows indicate developmental stages collected for extraction.*

The P-generation experienced LD or SD from the fourth larval stage. The G_1 generation was kept under either LD or SD conditions until adult. Part of the LD aphids were subjected to crowding conditions to obtain alatae. A batch of SD G_1 aphids was sacrified for analysis. Twelve days after final ecdysis the first sexuparae were produced.

- Analyses

Aphids selected for analyses were individually weighed and either decapitated in 0.05 ml of Levy-solution (154 mM NaCl, 9.5 mM KCL, 4.1 mM $CaCl_2$) to exclude embryo's or eggs, or homogenated in hexane. The composition of mono- and sesquiterpenes was determined according to Gut & Van Oosten (1985). The same procedure was followed for the fraction of haemolymph and symbionts. Section of the aphids provided an additional check for the selection of a certain morph for anatomical characters such as presence of pseudorhinaria on the hindlegs of oviparae.

RESULTS

The haemolymph of all aphid morphs contain both mono- and sesquiterpenes. This is in contrast with other species of Aphididae, such as *Myzus persicae*, in which only sesquiterpenes can be detected (Gut & Van Oosten, 1985). As Table 1 (or Fig. 2) shows, the highest amount of monoterpenes were found in apterous LD aphids and the presence of α-pinene, ß-pinene and limonene could easily be detected. The dominating sesquiterpene in *M. viciae* is (E)-ß-farnesene, although calculated on insect biomass this aphid species contains about one order of magnitude less than *M. persicae*. Minor amounts of (Z,E)-α-farnesene were also found in a ratio of 22:1, which is comparable with that in *M. persicae* (Gut & Van Oosten, 1985). The isomer (E,E)-α-farnesene could not be detected. Eggs did not contain any terpenes.

In LD reared aphids the haemolymph of alatae has a ten times lower content of the two farnesene isomers than that of apterae. There is also a reduction in the amount of monoterpenes, but it is limited by a factor 3-4.

Morphs	Monoterpenes ng / mg aphid			Sesquiterpenes ng / mg aphid		
	α-pinene	ß-pinene	limonene	(E)-ß-F	(Z,E)-α-F	(E,E)-α-F
apterae LD (1)	0.50	9.50	0.27	1.77	0.08	?
alatae LD (2)	0.14	2.96	0.07	0.17	0.007	?
apterae SD (3)	0.20	3.35	0.13	1.25	0.05	?
oviparae SD (4)	0.21	3.22	0.06	0.26	0.009	?

Table 1. Amounts of isomers of mono- and sesquiterpenes in different morphs of M. viciae. *Numbers between brackets refer to those in Fig. 1.*

Under SD conditions the amounts of monoterpenes in virginoparous adults of the G_1 generation is about as strongly reduced as in LD alatae of the G_2 generation but that does not hold for the farnesenes. Only the determination of the oviparous morph in the G_2 generation is marked by a sharp drop in farnesene content. SD G_1-adults however, are characterized by an almost equally strong drop in their monoterpene content as found in LD G_2 alatae. The changes of all three monoterpenes and of the farnesene isomers were of the same direction and value, indicating that a key enzyme regulates the synthesis of these compounds in aphids.

Although *M. viciae* is a relatively large aphid even the apterous morphs contain less sesquiterpenes than do comparable stages of *M. persicae* and *Macrosiphum euphorbiae* (Gut et al., 1991), in which the winged morphs have a considerably reduced amount of (E)-ß-farnesene.

About 50 mg of conditioned *M. viciae* were needed to enable estimation of (Z,E)-α-farnesene. In this biomass the isomer (E,E)-α-farnesene could not be detected.

DISCUSSION

Different environmental factors induce the production of winged virginoparae and of sexuparae. Wing dimorphism of virginoparae is governed by crowding and host plant factors, both variable with time. The production of sexuparae in temperate regions occurs when daylength comes below a critical value, which is a predictable fact. Possibly the neurosecretory system is involved in both types of regulation, although the role of neurosecretory cells in the protocerebrum and the activity of the corpus allatum (CA) could only be related to the induction of sexuparae (Hardie, 1987).

The amount of both monoterpenes and sesquiterpenes is related with polymorphism in *M. viciae*. Application of terpenoid end products strongly affects developmental processes in aphids (Gut et al., 1991). These facts, together with the proven function of terpenes as pheromones may serve to position mono- and sesquiterpenes among products of the mevalonate metabolism in aphids (Fig. 2). The synthesis of JH_{III}, found to be present in *M. viciae* and *A. fabae* (Hardie et al., 1985) is clearly related to that of sesquiterpenes. Hardie found the main titre of JH_{III} in LD *M. viciae* to be about three times higher as in SD reared aphids. Although the levels of JH_{III} appear to be a factor 10^{-3} less than those of terpenes, the difference between the levels of monoterpenes in LD and SD *M. viciae* is the same as found for JH_{III}. Hardie et al. (1985) estimated the amount of JH_{III} in whole aphids, while we analysed haemolymph. We found a relatively high amount of farnesenes in late embryo's of winged virginoparae and although the third larval instar is the most important one in the determination of adult characters (Hardie, 1987) the CA is already present before birth.

The considerable amount of terpenes found in aphids suggests that they are synthesized in other organs than the CA alone and the symbionts are a serious candidate for their production (Piron et al., 1991). The fact that both monoterpenes and sesquiterpenes are reduced under SD conditions indicates that HMG-CoA reductase (Fig. 2) is a key enzyme for their

synthesis in *M. viciae*. Differences in storage or release (symbionts, oenocytes, reproductive system) may explain why shifts in the level of monoterpenes and sesquiterpenes are not exactly the same. The ratio of

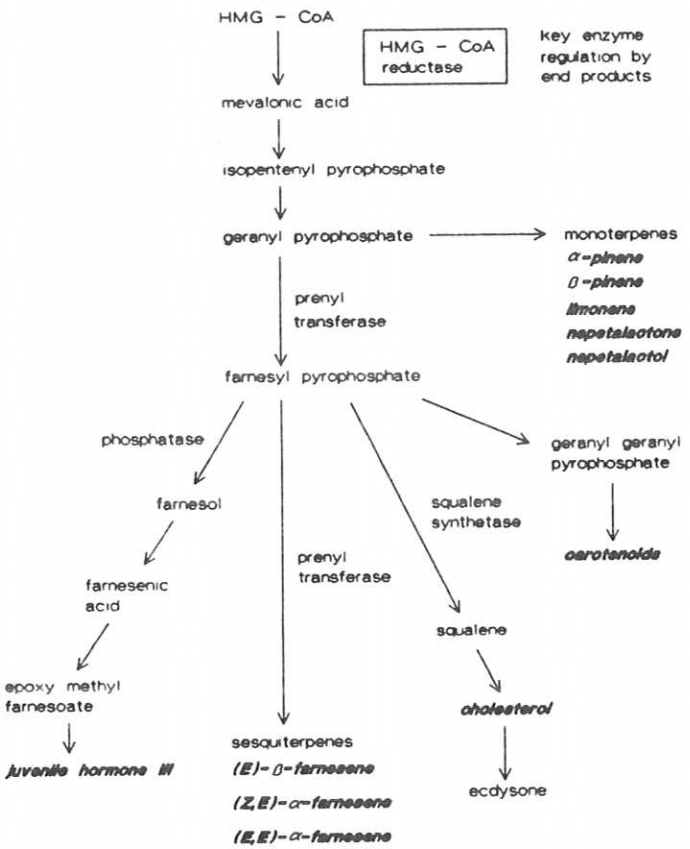

Fig. 2. Partly tentative scheme of mevalonate metabolism in the aphid M. viciae. *Compounds printed in bold italics are proven to be present.*

the different isomers, however, seems to have a constant value indicating that regulation may occur at the site of HMG-CoA reductase, a phosphatase or prenyl transferase (Fig. 2). When long photoperiods activate synthesis and release of JH_{III} key enzymes for the synthesis of monoterpenes and sesquiterpenes could also be activated. CA regulating substances may well act via HMG-CoA reductase (Khan, 1988). The subsequent determination of alateness is induced by other external factors that have an impact on a different site of the neurosecretory system (Harrewijn, 1976). The next generation (G_2 in Fig. 1) consists of winged virginoparae with a strongly reduced farnesene synthesis (Table 1 or Fig. 2) suggesting that the differentiation of wing buds and flight muscles is linked to inhibition of a prenyl transferase at other sites than the CA.

Winged virginoparae, at least in their early reproductive life, give birth to apterous offspring with high activity of HMG-CoA reductase, active DNA synthesis in the reproductive system and suppression of wingbud differentiation.

It is remarkable that mevalonate metabolism can create a variety of biologically active compounds in plants, insects and animals ranging from fatty acids to hormones, the activity of which is regulated in aphids by specific end products depending on the site of their synthesis or release.

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THE VISUAL WORLD OF THE COLORADO POTATO BEETLE

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Keywords:
Leptinotarsa decemlineata, vision, spectral sensitivity, host-plant finding.

Summary. The visual capabilities of the Colorado potato beetle were studied. A locomotion compensator was used to record behavioural responses. The beetles were highly attracted by a yellow paper stripe compared to a grey field. Individual objects were discriminated by Colorado potato beetles on their size and larger ones are preferred. Resolving power of Colorado potato beetles was about 2°. Field crops of 30 cms high can be detected by the beetle at eight meters or less. The beetles made no choice between two equally-sized stimuli until the stimuli became separated more than 90° apart. The orientation based upon visual discrimination of plant characteristics on a distance will increase the probability of host-plant finding by the beetle.

INTRODUCTION

Human beings do have a well developed visual system which is important to cope with every day life. The present question is how insects depend on vision. The Colorado potato beetle *Leptinotarsa decemlineata* Say, a pest species on potatoes, is able to locate food as well as conspecifics for vital processes such as growth and reproduction. At present it is difficult for us human beings to understand how the beetle's perceptual world is like during host-plant finding. For this reason we studied several aspects of the visual world of the Colorado potato beetle.

MATERIAL AND METHODS

Beetles

Newly-emerged female Colorado potato beetles were obtained from the laboratory stock culture and isolated in petri dishes lined with wet filter paper. Prior to the experiments the beetles were fed for four hours on pieces of potato leaves and subsequently starved for at least 16 hours. At the time of the experiments all females were about one day old. The

beetles were reared and tested under long-day (16/8) light conditions.

Locomotion compensator

The experiments were conducted on a locomotion compensator as described previously (Thiery & Visser, 1986). Light intensity was set at 1300 Lux by means of two high-frequency illumination units (1750Hz). A white paper arena (diameter of 48 cms, height of 24 cms) was placed around the top of the locomotion compensator and visual objects were attached at the inside wall. Yellow ochre paper was used as a stimulus because of its attractiveness to the beetles. After each test the stimulus was moved +90° to abandon effects of orientation other than visual. Behavioural responses of the beetles were studied by recording their walking tracks on the locomotion compensator for successive treatments. Mann-Whitney *U* tests (two-tailed) are used for making statistical analyses (Siegel, 1956).

RESULTS AND DISCUSSION

Color vision

In preliminary experiments, it has been found that Colorado potato beetles were attracted by grey and black objects displayed on a white background. In the next experiment color attraction of Colorado potato beetles, *i.e.* spectral sensitivity in competition with contrast sensitivity, was examined. Two equal bright stimuli in terms of photographic contrast, a color neutral one (grey) and the yellow ochre paper, were matched. Results are shown in figure 1.

No significant decrease in directional response was found for beetles exposed to a yellow ochre stripe on a background being grey instead of white. The cosine of the walking direction was found to be significantly decreased ($P < 0.0001$) for beetles walking to a grey field in absence of the yellow stripe. In this latter treatment the beetles showed orientation towards the edges of the grey field which caused the distribution of orientation angles show a dip at 0° (fig.1).

From these results it is obvious that Colorado potato beetles are attracted by color rather than by contrast. Spectral sensitivity of Colorado potato beetles has previously been examined by measuring ERG-potentials (Mischke, 1981). Two maxima in responses were found at 360 nm (ultraviolet) and 510 nm (green). Stüben (1972) showed that Colorado potato beetles were able to distinguish between colors of the same light-intensity and preferred yellow. It is likely that color vision assists Colorado potato beetles in finding their host plants.

Resolving power

The size of an object at a certain distance or the distance to an object of a certain size determines the ability of Colorado potato beetles to perceive the visual information. The dimensions of a visual stimulus are characterized by the angle of perception of the object by the beetle's

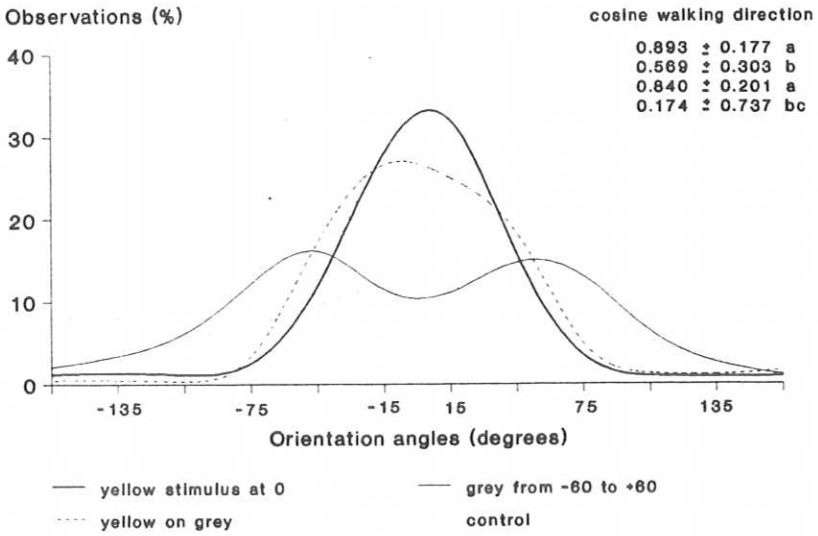

Fig. 1. Orientation of 20 beetles in 10 minutes each to: (1) yellow stripe at 0°, (2) grey field covering -60° to +60°, (3) yellow stripe on grey field, and (4) control. Seconds spent at orientation angles as % observations. Cosines of directions (mean and SD) from top downwards: experiments (1)-(4). Different letters indicate P≤0.05.

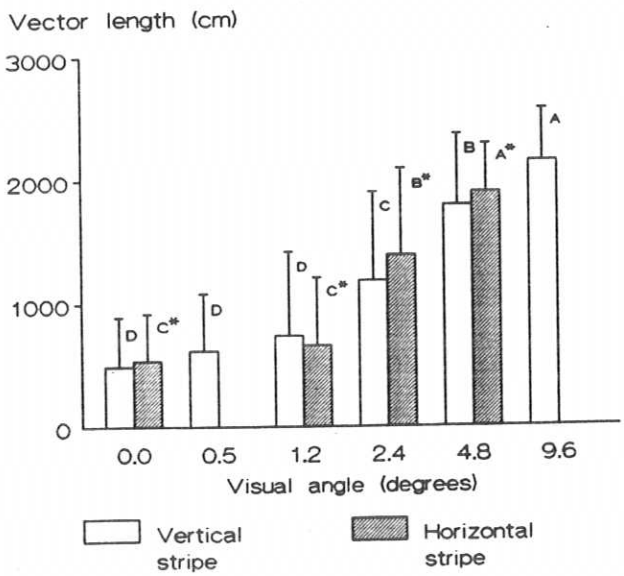

Fig. 2. Displacement from the origin (vector length) of 20 beetles in 5 minutes each to yellow stripes. Dimensions of stripes are expressed as visual angles in the beetle's eye. SDs shown half. Different letters indicate P≤0.05.

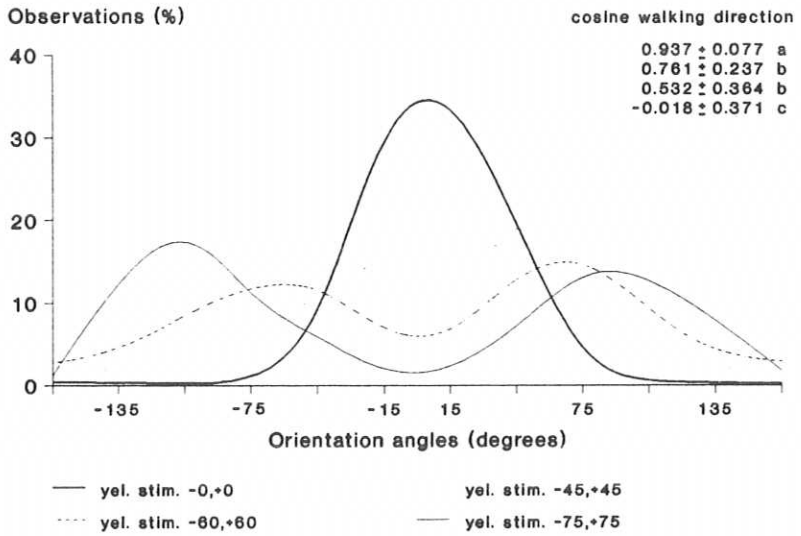

Fig. 3. Orientation of 20 beetles to 2 yellow stripes. Position of stripes: (1) -0/+0, (2) -45/+45, (3) -60/+60, and (4) -75/+75. Cosines of walking directions (mean and SD) from top downwards: experiments (1)-(4). See further fig. 1.

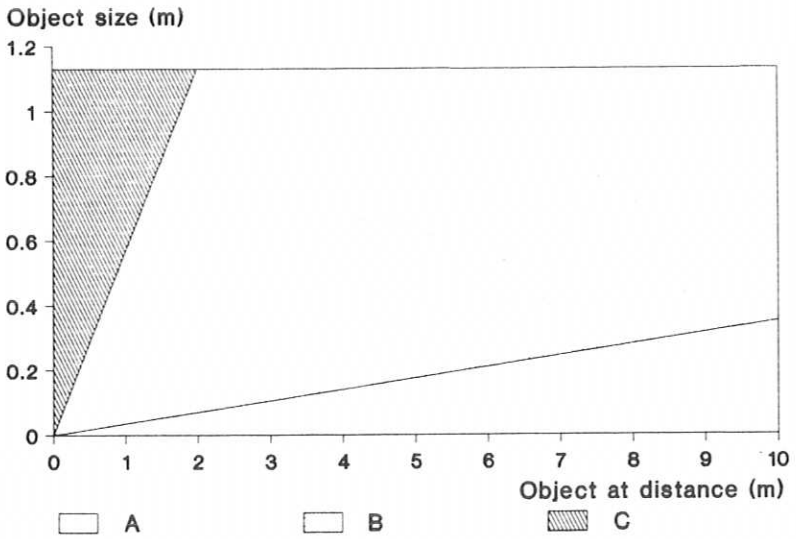

Fig. 4. Depending on the size of an object and the distance: (A) object is too small for perception by the beetle, (B) perception and discrimination in size are possible, and (C) perception is possible, discrimination in size is not possible.

compound eye. The minimum angle of perception to evoke a directional response, which thus is related to the minimum size of an object at a certain distance, is called resolving power. The resolving power of Colorado potato beetles was examined by displaying vertically or horizontally a yellow ochre stripe and decreasing the size of the stripe in successive treatments. Results are shown in figure 2.

The smaller the object the more difficulties the beetles encountered to respond to that stimulus. When the vertical or horizontal dimensions of the yellow ochre stripe became 1.2° or less the objects became too small for perception by the beetles. In this case the net displacement was not significantly different from the control.

The resolving power of Colorado potato beetles appeared to be about 2°. This value has been found for several other insects as well, for example *Musca* (Kirschfeld, 1973) and *Lymantria dispar* (Preiss & Kramer, 1984).

Visual interference

In field situations Colorado potato beetles are usually confrontated with more than one object. The beetles do have to make choices. Competition between two unequally-sized stimuli resulted in preference for the largest stimulus (unpubl., L.J. van der Ent). In the present experiment two equally-sized yellow ochre stimuli were given and the distance between these objects was enlarged in successive treatments. Results are shown in figure 3.

On enlarging the distance between both stimuli Colorado potato beetles were observed to walk zigzag instead of rectilinear . Obviously, the beetles were switching between both yellow ochre stripes for direction keeping, especially when both stimuli were displayed at -45° and +45°. The directional response at this latter treatment was significantly lower compared to the ±0° and ±15° treatments ($P=0.0036$ and $P=0.0008$, respectively). The cosines of orientation angles at the ±15° and ±30° treatments were not statistically different from the ±0° treatment. In the two last treatments the beetles showed attraction to one stimulus at the time. Half of the beetles walked to the stimulus displayed left and the other half to the stimulus at the right side. The distribution of orientation angles, thus, showed a dip at 0° (fig.3). In the absence of stimuli, the control experiment, the cosine of the orientation angle was -0.052 ± 0.694 (mean and SD).

This so called 'moment of decision' at 90° distance is also reported for the Gypsy moth *Lymantria dispar* (Preiss & Kramer, 1984). However in their experiment Gypsy moths were exposed to a single stripe or field. Exposure of the beetles to a grey field covering -60° to +60° (fig.1) or yellow stimuli exposed at -60° and +60° (fig.3) resulted in similar distributions of orientation angles.

Host-plant finding

In the present research Colorado potato beetles were able to discriminate a yellow stripe from a grey background. The beetles responded to a single

object if the angle of perception of such object was at least 2°. The minimum angle of perception to discriminate between two equally-sized objects appeared to be 90°. Figure 4 illustrates further the visual capabilities of Colorado potato beetles. From the data in figure 2, it is calculated that a 30 cms-high potato crop, can be seen by the beetle at 8 meters distance or less. However, it should be realized that laboratory conditions differ from the outside world and, thus, may have affected the visual perception as recorded in the present study. On the other hand, field-reared beetles do have more sensitive eyes (unpubl., J.H. Visser). Moreover, the spatial distribution of food resources will be more complex than assumed in the present research. In addition to visual orientation, olfactory stimuli are perceived by the beetles (Thiery & Visser, 1986). The interplay of both sensory modalities will affect host-plant finding in complex and yet unknown ways. Nevertheless, it is obvious that Colorado potato beetles are capable to perceive the surrounding world and on perception of visual characteristics increase their probability of encountering host-plants.

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PEPTIDERGIC NERVE CELLS DELIVER MULTIPLE MESSENGERS FOR OPTIMIZING NEUROENDOCRINE COORDINATION IN THE COLORADO POTATO BEETLE

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Keywords:
Colorado potato beetle, *Leptinotarsa decemlineata*, nervous system, peptidergic neuron, immunohistochemistry.

Summary

The question is raised as to how insects manage to control complex behavioral and physiological precesses with a nervous system that contains relatively few neurons. The explanation put forward here is that many neuronal and neurohormonal functions are combined in specific (peptidergic) neurons.

INTRODUCTION

The problem of small size

Insects have to cope with the problem of their small size. Like the 'higher' animals, their nervous system controls diverse functions such as maintenance of homeostasis, handling of reflexes, numerous forms of behaviour, learning, and so on. But the insect nervous system has to do all this in much less space and with a much smaller set of neurons than in the nervous system of higher animals. The total number of neurons in a medium-sized insect such as the housefly is rather low (about 338.000) in comparison to vertebrates (10 billion) (Strausfeld 1976). How then can all functions be executed?

Insects have 'developed' various ways to overcome dimensional problems:

a. Various types of behaviour are in fact programmed as 'stereotyped' sequence of actions. That means, a limited set of neurons, requiring only little space, are linked efficiently to control a fixed set of muscles in a fixed order.

b. Several types of neurons are built relatively complex. They have well-branched dendritic webs to 'listen' to instructions from a wide variety of neurons in different places. On the other hand, their axons are also extensively branched, such as to convey messages to many neurons 'listening' to them. In this way, insect neurons communicate with large numbers of other neurons and innervate several target organs.

c. A certain number of -mostly large- neurons, specialized in the synthesis and release of peptide messengers, communicate with their targets in two essentially

different ways: (1) Via nervous communication: neuropeptides are released in or near synaptic clefts, either within the central nervous system (CNS) or in peripheral target organs. Their function is to activate receptors in postsynaptic target cells, situated in -or close to- the cleft. (2) Via the blood stream as true peptide hormones. The effect is that one neuron is in essence able to control a multiplicity of physiological processes by delivering its messengers at several specific places.

d. Certain motor neurons acquire different functions in the course of development and metamorphosis by rearranging their synaptic contacts.

e. Certain neurons are able to generate more than one type of messenger substance, and thus acquire the competence to address receptors on different sets of target organs.

All these specializations, few of them by itself specific for insects, make that the neuronal circuitry can control body functions effectively due to neuron flexibility and in spite of their small dimensions. The last-mentioned specialization, the colocalization of messenger substances, will be further illuminated.

The phenomenon of colocalization of neuronal messengers in insects was demonstrated for the first time for proctolin and L-glutamate in *Periplaneta americana* by Adams and O'Shea (1983). It was demonstrated in the Colorado potato beetle that the peptides -or at least related substances- FMRFamide, vasopressin, and -MSH were colocalized in the same neurons in the suboesophageal ganglion (SOG) (Veenstra 1984).

Table 1. *Demonstration of differentially colocalized neuropeptides with 2 monoclonal antibodies (MAC-3 and MAC-13) and a polyclonal antiserum against FMRFamide (#544, courtesy Dr. C.J.P. Grimmelikhuijzen) in selected neurons in different ganglia. Positive immunoreactions are indicated with the "+" sign.*

	MAC-13	MAC-3	FMRFamide
Pars intercerebralis A-type	+	-	-
C-type	-	+	+
Suboesophageal ganglion (3)	+	+	+
Thoracic ganglia type 1	+	-	+
type 2	+	-	-
type 3	-	-	+
type 2	+	-	-
type 3	-	-	+
Abdominal gangl type 1	+	-	-
type 2	-	-	+
Frontal ganglion (6 neurons)	+	-	+

Table 2. *Summary of immunoreactions of selected types of peptidergic neurons, obtained with MAC's and polyclonal antisera against a variety of antigens, in addition to the data in Table 1. Antigens are given in quotes if they have not been fully demonstrated in this species. Asterisks indicate that colocalizations have to be confirmed by double-labelling.*

Corpus cardiacum: intrinsic glandular cells (2 types)	Adipokinetic hormones: Myogenic factors I, II 'vasopressin' 'oxytocin'
Frontal ganglion	'FMRFamide' MAC-13 antigen serotonin *
Lateral NSC	'FMRFamide' 'oxytocin-2' 'prolactin' MAC-18 antigen
Medial NSC (A- or A1 type)	'\ and ß-endorphin' 'enkephalin' 'calcitonin' 'growth hormone' 'insulin' MAC-7 antigen
(E-type)	'gastrin releasing peptide 'cholecystokinin'
Suboesophageal ganglion (3)	'FMRFamide' proctolin * 'PBAN' * MAC-13 antigen MAC-3 antigen

METHODS TO DEMONSTRATE COLOCALIZATION

The demonstration and localization of messengers can in most cases be done conveniently by immunohistochemical methods. It is essential that antisera or monoclonal antibodies are available which specifically recognize the messengers in microscopical preparations, usually paraffin, epon, or cryotome sections. Such antibodies recognize the messenger substance. The formation of this immune complex can be visualized with secondary antibodies conjugated to markers such as (1) peroxidase, an enzyme converting the substrate 3,3'-diaminobenzidine (DAB) into an opaque brown precipitate and (2) small gold spheres associated with the secondary antibody to be studied in the electron microscope (EM).

The demonstration of colocalization requires a double-staining technique that is slightly more complex (Schooneveld and Veenstra 1988). Most neurons are so large that two or more sections can be cut through it. Each section is mounted on a separate glass slide and is processed with one specific antibody. If the neuron is traced in

each section afterwards, colocalized antigens can be demonstrated by superimposing their images.

RESULTS

Cases of colocalization in the Colorado potato beetle

Making use of certain recently prepared monoclonal antibodies anti-Colorado potato beetle (i.e. MAC-3 and MAC-13) (Schooneveld et al. 1989) and a polyclonal antiserum against FMRFamide (Triepel and Grimmelikhuijzen 1984), we investigated whether antigens were colocalized in peptidergic neurons that can easily be recognized in both EM and light microscope (LM). These neurons are localized in: (1) the pars intercerebralis of the protocerebrum; (2) three groups in the ventral

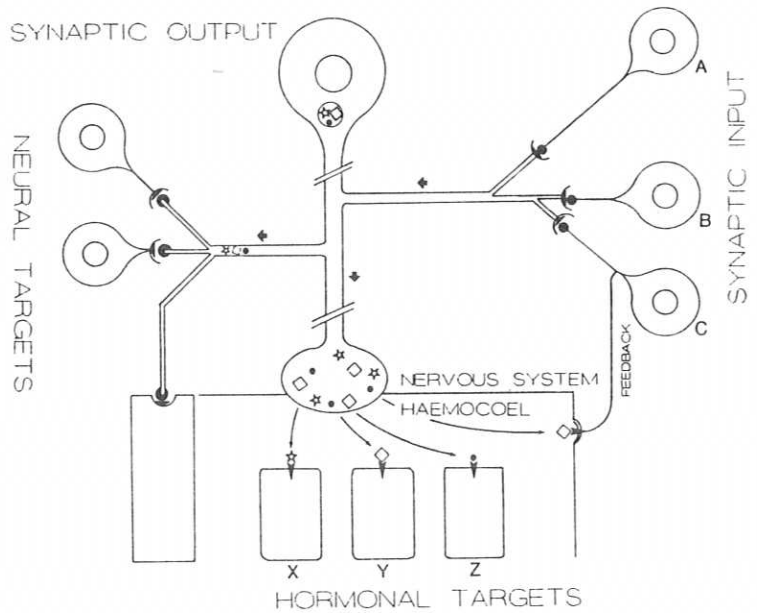

Fig. 1. Highly diagrammatic representation of a hypothetical peptidergic neuron with several colocalized neurally or hormonally active principles. The neuron is influenced synaptically by several other neurons (A, B) and by C, representing a hypothetical feedback loop. Messages are send out to other neurons in the CNS or to neuronal targets outside the CNS, and to a set of hormonal target organs (X, Y, Z) addressed through the bloodstream. The messages are received only if targets are equipped with receptors for a particular messenger substance. Different antigens in the central neuron may be actually colocalized in the same secretion granule (not shown).

part of the suboesophageal ganglion comprising 4, 8, and 2 neurons, respectively; (3) the thoracic ganglia; (4) the fused abdominal ganglia; (5) the frontal ganglion.

Using LM and EM double-labelling methods, we obtained the results summarized in Table 1. The data actually represent an excerpt of a larger screening program (Schooneveld et al. 1991) indicating that immunoreactive substances are present also in other parts of the central and peripheral nervous system. The point to be made here is that the antigens occur in specific combinations, depending on the type of neuron. The neurons in the SOG are unique in that they contain all 3 antigens. Those in the C-type and A^1-type neurons in the brain, certain thoracic and all abdominal ganglia contain only one antigen. Others contain various combinations. We do not know, in this phase, what the nature and physiological action of the immunoreactive substances is. At any rate, the substances in the pars intercerebralis and SOG neurons are released from the corpora cardiaca, probably to act as hormones elsewhere in the body. Different types of neurons obviously deliver different mixtures of messengers.

The complexity of signals may still be greater. In our staining protocols collected over the years and using antisera to a wide variety of peptides of different origin, i.e. most 'vertebrate' peptides, the phenomenon of colocalization is common for many more substances. Although the criteria for colocalization have not been studied as thoroughly as in the above-mentioned experiment, the other types of neurons most likely contain multiple messengers, as listed in Table 2. Again, the neurons in the SOG, already mentioned, appear to react also with antisera to proctolin, -MSH, and PBAN. It was also unexpected that the intrinsic secretory cells in the CC contain more substances than the two AKH-related peptides. So far, vasopressin/oxytocin-related substances have not been isolated from CC of any insect species but these data indicate that it might be worth looking into this possibility.

DISCUSSION

There is little evidence that the used antisera detect the authentic antigens. The possibility of cross-reactions with unrelated substances. just sharing one epitope with the antigens, is very real. To what extent these substances are the same that react with our monoclonal antibodies, remains uncertain as well. A peptide isolation program is in progress to characterize some of the substances that seem relevant for a better understanding of endocrine control mechanisms.

The hypothesis we favor at this time is that these peptidergic neurons- and possibly other neurons not taken into consideration here- are equipped to release a multitude of peptidergic messengers and at different places and perhaps at different

times (Fig. 1). Different targets are addressed through the bloodstream. Other, neuronal, targets are addressed through the strongly ramified axon collaterals in the neuropil. If we consider that each peptidergic neuron also receives information from different places, including from neurons that occupy a position in a feedback system, we realize that just one neural unit can play a highly important role in neuroendocrine pathways. Only few of these complex neurons are required for complex functions and we can see them as a highly sophisticated communication centers, in spite of their small dimension. Much work remains to be done to analyze the nature of their chemical messengers and the sort of physiological process they are involved with.

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THE FUNCTIONAL SIGNIFICANCE OF ENHANCED ENDOCYTOSIS IN FLIGHT-STIMULATED ADIPOKINETIC CELLS IN THE CORPUS CARDIACUM OF *LOCUSTA MIGRATORIA*

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Keywords:
Locusta migratoria, corpus cardiacum, adipokinetic cells, endocytosis.

Summary

In flight-stimulated as well as in non-stimulated adipokinetic cell bodies, endocytosis is coupled mainly to uptake of nutritional or regulatory substances, whereas in the cell processes, endocytosis rather compensates for exocytosis.

INTRODUCTION

The adipokinetic cells (AKCs) in resting, unflown locusts show a remarkable endocytotic activity (Jansen & al.,1989). Their exocytotic activity, however, is very low, because of the absence of flight activity. Flight is the only known natural stimulus for the exocytosis of adipokinetic hormones (AKHs) from the AKCs. This suggests that in the AKCs of resting locusts, endocytosis is coupled mainly to uptake of nutritional or regulatory substances rather than being compensatory for exocytosis. Coupling between endo- and exocytosis may be expected to be prominent in flight-stimulated AKCs, which are actively releasing AKHs by exocytosis. In order to get more insight into the functional significance of endocytosis for the AKCs, an ultra-structural morphometric study was made of the endocytotic system in non-stimulated and in flight-stimulated AKCs.

MATERIAL AND METHODS

Adult male locusts (*Locusta migratoria*) were used 12 days after imaginal ecdysis. The influence of flight was tested by suspending locusts on a motor-driven roundabout for 60 min; controls were kept resting during that period. The endocytotic pathway was revealed by injecting an endocytotic tracer (HRP or WGA-HRP) into the locusts. **Experiment I**:HRP injection, 10 min rest, 60 min flight. **Experiment II**:60 min flight, HRP injection, 10 min rest. **Experiment III**:WGA-HRP injection, 6h rest, 60 min flight.

After decapitation, the corpora cardiaca with the AKCs were fixed with a glutaraldehyde/formaldehyde mixture, the (WGA)HRP was visualized enzyme-cytochemically, and the tissue was post-fixed in OsO4 and prepared for transmission electron microscopy, after routine procedures.

The numbers of the various categories of tracer-containing organelles were manually counted on electron-microscopic photographs. Parameters indicative of the amount of endocytosed (WGA)HRP were measured on the photonegatives by means

of an automatic image analysis system. Numbers of endocytotic pits and vesicles were expressed per unit of plasma membrane length, and the other parameters were expressed per unit of cytoplasmic area of the cell bodies and processes analysed.

RESULTS AND DISCUSSION

In exp.I, the numbers of endocytotic pits and various intracellular endocytotic and lysosomal organelles containing HRP had markedly increased in both the cell bodies and cell processes of the flight-stimulated AKCs, as did the total amount of HRP taken up by these cells. This indicates that endocytosis was clearly stimulated by flight.

Similar results in exp.II indicated that the stimulatory influence of flight on endocytosis continued for some time after cessation of flight, for HRP became available to the AKCs not earlier than after cessation of flight.

In both exp.I and II, a flight-induced increase in the number of labelled endocytotic pits in the cell processes was observed. The increase in endocytotic activity in the flight-stimulated cell processes, therefore, may be considered to be a form of adaptive endocytosis, which compensated for membrane material added to the plasma membrane during flight-induced exocytotic release of AKHs from the cell processes.

In exp.II, a flight-induced increase in the number of labelled transfer tubules was observed in the cell bodies, these tubules being practically absent in the processes. Transfer tubules rapidly return to the plasma membrane membrane material that just had been endocytosed (Van Deurs & Christensen,1984). The increase in endocytotic activity in the flight-stimulated cell bodies, therefore, may be considered to be a vehicle for an increase in the uptake of nutritional or regulatory substances.

In flight-stimulated as well as in non-stimulated AKCs of exp.III, the endocytotic tracer WGA-HRP was found in the trans-Golgi network (TGN) and in some scretory granules originating from it. This indicates that the TGN is linked to the endocytotic system and takes part in the recycling of membrane material back to the plasma membrane via the secretory route. This linkage apparently can be shown only by the adsorptive tracer WGA-HRP and not by the fluid phase tracer HRP, which did not appear in the TGN. Flight activity had no detectable influence on the degree of labelling of the TGN and the secretory granules originating from the TGN. This suggests that flight activity does not influence the production of secretory granules.

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A NEW, THIRD, ADIPOKINETIC HORMONE FROM THE MIGRATORY LOCUST, *LOCUSTA MIGRATORIA*

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Keywords:
New adipokinetic hormone (Lom-AKH-III), *Locusta migratoria*, locust.

It is now well established, that in locusts adipokinetic hormones (AKHs) are important for the mobilization of energy substrates from the stored reserves in the fat body to meet the demands of the flight muscles. From the migratory locust, *Locusta migratoria*, two different AKHs are known: a decapeptide Lom-AKH-I (see for nomenclature Raina & Gäde, 1988) (Stone *et al.*, 1976) and an octapeptide Lom-AKH-II (Siegert *et al.*, 1985). Both hormones are synthesized and stored in intrinsic cells of the glandular lobes of the corpus cardiacum (CC) (Oudejans *et al.*, 1990). Two prohormones are involved, which form dimeric precursor molecules via a disulfide bridge and after proteolytic processing result in the bioactive hormones and dimeric AKH-related peptides (Hekimi *et al.*, 1989; Oudejans *et al.*, 1991).
In this paper we report on the isolation and identification of a third adipokinetic hormone (Lom-AKH-III) from *L.migratoria*.

Lom-AKH-I	pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2
Lom-AKH-II	pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp-NH_2
Lom-AKH-III	pGlu-Leu-Asn-Phe-Thr-Pro-Trp-Trp-NH_2

The peptides from the glandular lobes of the CC were extracted with 1% acetic acid in 90% methanol or with 0.1% trifluoroacetic acid and separated by reversed phase HPLC. Apart from the AKH precursor peptides, AKH-I and II and their dimeric related peptides, an apparently new adipokinetic peptide with strong adipokinetic activity was found using an appropriate bioassay (Goldsworthy *et al.*, 1972). The unknown adipokinetic peptide was isolated from about 700 glandular lobes and subjected to fast atom bombardment mass spectrometry (FAB-MS).
In this way the complete chemical structure of the adipokinetic peptide was established (Oudejans *et al.*, 1991), which was supported by Edman degradation of the pGlu-deblocked peptide. The structure of the new peptide was confirmed by chemical synthesis via the solid-phase technique. All characteristics from HPLC, FAB-MS and biological activity of the natural and synthetic peptide appeared to be identical.

In vitro incubations of glandular lobes with several radiolabelled amino acids revealed that the biosynthesis of the new peptide is very fast and since no obvious accumulation of radiolabel was found after a long incubation time (17 hr), the new peptide has a high turnover or a fast release.
All evidence from its complete chemical structure, its adipokinetic action and its release by a high [K^+] containing medium strongly supports that the adipokinetic peptide is a new adipokinetic hormone with a unique very hydrophobic nature. It is the first member of the AKH/RPCH hormone family with a Trp in the 7-position. Its amino acid sequence suggests a biosynthetic route which is different from that for Lom-AKH-I and II. The glandular part of one corpus cardiacum of *L.migratoria* contains 15-20 pmol of the new hormone. It is not found in the storage part of the CC and, interestingly, also is not present in another locust species, *Schistocerca gregaria*.

The physiological role of three different adipokinetic hormones in one species is intriguing and further experiments are in progress to solve this question.

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THE INNERVATION OF THE ADIPOKINETIC CELLS IN THE CORPUS CARDIACUM OF *LOCUSTA MIGRATORIA*

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Keywords:
Locusta migratoria, corpus cardiacum, adipokinetic cells, octopamine.

Summary

Immunocytochemistry with an antiserum raised against octopamine, direct binding studies with [3H]octopamine as a radioligand, and measurements of released adipokinetic hormone I after incubation of isolated corpora cardiaca with octopamine do not indicate octopamine to be involved in the release of adipokinetic hormone from the adipokinetic cells.

INTRODUCTION

In insects, the glandular part of the corpus cardiacum (CC) is the site for synthesis and release of the adipokinetic hormones (AKH) I and II. Data from literature suggest that secretomotor cells in the lateral part of the protocerebrum regulate the activity of the adipokinetic cells via the nervus corporis cardiaci (NCC) II with the use of octopamine (OA) as a neurotransmitter.

MATERIAL AND METHODS

With an antiserum raised against OA sections of brains and CCs of adult locusts (*Locusta migratoria*), 12 days after imaginal ecdysis, were immunostained using the peroxidase-antiperoxidase method. The metathoracic ganglion was processed as a control tissue because of the presence of dorsal unpaired median (DUM) neurons known to contain considerable amounts of OA.

The presence of binding sites for OA in membrane fractions from brain and CC was investigated using 3H-OA as radioligand. The pharmacological properties of the OA-binding sites were characterized using OA agonists and antagonists as competing ligands. The results were compared with data on OA-binding sites from literature.

Moreover, OA was tested for its ability to induce the release of AKH I from isolated CCs. Pools of CCs were incubated twice in succession. After an incubation in insect saline, the incubation medium was replaced either by saline provided with the substance to be tested (OA, high potassium) or by pure saline as a control. High potassium aspecifically stimulates isolated corpora cardiaca to release large amounts of AKH I into the incubation

medium. Release of AKH I into the incubation media was detected with reversed-phase high-performance liquid chromatography (RP-HPLC).

RESULTS AND DISCUSSION

The DUM neurons were found to be strongly OA-immunoreactive. Control reactions with antiserum preadsorbed with octopamine or related amines confirmed the specificity of the immunoreaction. OA could not be shown to occur in the dorsolateral part of the protocerebrum where the somata of the secretomotor neurons are located that innervate the adipokinetic cells of the CC. Also the NCC-II, through which the axons of these neurons extend in the glandular part of the CC, and the CC proper were immunonegative.

Incubation of membrane fractions from brains with increasing amounts of [3H]OA indicated saturable binding at radioligand concentrations of 100 nM and above. Scatchard analysis of the binding data suggested the presence of a single binding site. Displacement data of OA agonists and antagonists competing for binding, indicated that the binding site for OA is likely an OA-2A receptor. With membrane fractions of CCs, however, no specific binding could be detected.

The difference between the amounts of AKH I spontaneously released during the first and second incubation in saline, respectively, was used as the control value and was compared with the difference between the amounts of AKH I released during the first incubation in saline and the second incubation with OA or high potassium, respectively. High potassium strongly stimulated the release of AKH I, probably by depolarisation of the secretory axon terminals innervating the adipokinetic cells or by the secretory terminals of the adipokinetic cells proper. OA, however, did not have significant effects on the release of AKH I into the incubation medium.

On account of the results presented above, OA is not likely to be present as a neuroactive substance in synapses that innervate the adipokinetic cells.

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HUMORAL RESPONSE OF THE HONEYBEE (*APIS MELLIFERA* L.) IN RELATION TO *NOSEMA APIS* ZANDER

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Keywords:
Apis mellifera, Nosema apis, apidaecin, abaecin, humoral respons, survival rate.

Summary

Honeybees show a basic antibacterial activity in hemolymph. In addition they possess a humoral immune system, which is based on the release of antibacterial peptides. Nosema infection does not enhance the natural present antibacterial activity within 7 days post-infection (p.i.), neither does it induce humoral immunity. This was concluded after examining hemolymph from healthy, Nosema infected and bacteria immunized bees by growth inhibition tests and RP-HPLC analysis. Further the effect of immunization on the survival rate of Nosema diseased bees was described.

INTRODUCTION

The humoral response of several insect species to bacterial or parasite infection has been described (reviewed by DUNN, 1986). This immune response involves secretion of a variety of antibacterial polypeptides into the hemolymph. In honeybees a number of humoral factors have been identified and characterized: apidaecins and abaecins (CASTEELS et al., 1989; 1990).

In the study of the inflammatory response of insects to Microsporidia infection most emphasis was placed on the cellular reaction of the host (LAIGO & PASCHKE, 1966; BROOKS, 1971). Only recently, CRAIG et al. (1989) reported the

induction of several components in the hemolymph of Nosema apis infected honeybees. Although no antibacterial activity could be observed, the presence of apidaecin I and II was demonstrated in one out of five individual bees by mass spectrometric analysis.

In this paper the presence of humoral peptides in Nosema diseased bees was examined by a bacteria growth inhibition test and Reversed Phase - High Pressure Liquid Chromatografy (RP-HPLC). Further, the effect of immunization on the survival of Nosema infected honeybees was studied.

MATERIALS AND METHODS

Worker honeybees were collected from a broodcomb every 24 hrs and treated for seven days by standard method (JACOBS, 1979). Bees were immunized (day 6) or infected orally with N. apis (day 8) or both. Immunized bees were obtained by pricking the insects abdominally with a microcapillar contaminated with Escherichia coli bacteria (NCTC 9001). For Nosema infection, spores were added in the sugarsolution reaching a final concentration of $5x10^7$ spores/ml. Short-term experiments with Nosema infected bees demanded individual feeding with 10^6 spores. The insects were bled by puncturing the abdomen with a glass capillary and samples from 5 bees were pooled.

Antibacterial activity was assayed by measuring growth inhibition of E.coli (NCTC 9001) or Micrococcus lysodeïkticus (LMG 4050) in thin agar plates.

RP-HPLC was performed on a ABI 150A system (Applied Biosystems Inc., Ramsey, NJ) with a Vydac C4 (214TP54) analytical column (The Separations Group, Hesperia, CA). Hemolymph samples were heat-treated (100°C, 5 min) and the precipitate was spun down. Clear supernatant (20 µl) was acidified by addition of 700 µl 0.1 % trifluoroacetic acid (TFA) before loading. Solvent A was 0.1 % TFA (pH 2) and solvent B: 70 % acetonitrile (MeCN) in A. Fractions were eluted at 1 ml/min with a two-step linear gradient: 0-50 % B/25 min, 50-100 % B/12 min. UV-detection was done at 214 nm.

The survival tests were done in triplicate at 34°C and 60 % relative humidity.

RESULTS AND DISCUSSION

Results of the growth inhibition tests are shown in Tabel 1. In accordance with MOHRIG & MESSNER (1968), it seems that blank bees have a basic antibacterial activity in the hemolymph, which is effective against M. lysodeïkticus. Nosema infection doesn't influence this natural present level within 7 days p.i.. Blank bees have no antibacterial activity against E. coli, neither do Nosema infected bees. Since it was demonstrated elsewhere (CASTEELS et al., 1989; 1990) that apidaecins and abaecins are highly effective against E. coli (NCTC 9001) it seems that there is no induction of these humoral peptides during Nosema disease.

	Nosema apis infected											Bl	Imm
Time p.i.(hrs):	12	24	36	48	60	72	84	96	108	120	168		
E.coli	-	-	-	-	-	-	-	-	-	-	-	-	+++
M.lysodeïkticus											+	+	+++

Tabel 1: Growth inhibition test: Bl = blank bees; Imm = immunized by injection of bacteria; - = no inhibition; + = low inhibition; +++ = strong inhibition

RP-HPLC results (Fig.1) confirmed these data: Nosema infection does not activate the production of apidaecin I and II, nor abaecin. In comparison with blank hemolymph we could notice an increase or decrease of some peaks in the HPLC patterns of Nosema diseased bees. This might be caused by 1/ the physiological reaction of the the host on the infection and (or) 2/ depletion of the protein content of the hemolymph. The absence of a humoral response possibly reflects the restriction of parasite presence to only the midgutepithelium (de GRAAF & JACOBS, 1991) and the omission

of secundary bacterial infections. In this context hemolymph of Nosema infected bees (untill 120 hrs p.i.) was inoculated on nutrient agar plates and no bacteria could be detected.

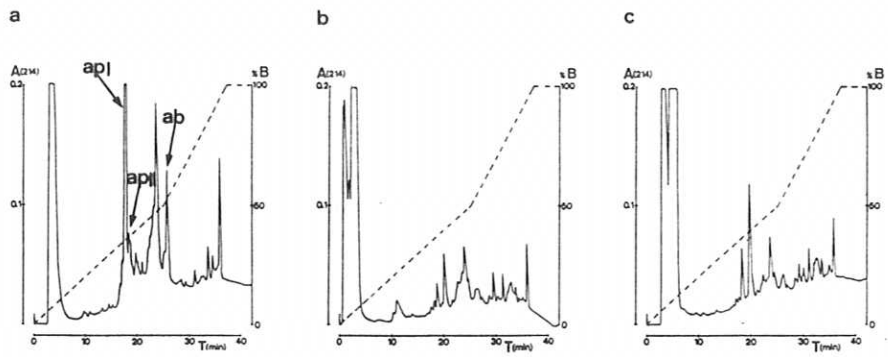

Fig.1: RP-HPLC patterns of heat-treated hemolymph from a) bacteria immunized, b) Nosema infected (7 days p.i.) and c) blank honeybees; apI = apidaecin I; apII = apidaecin II; ab = abaecin

The survival tests demonstrated the increase of mortality by Nosema infection. However, immunization prior to feeding the bees with spores, does not improve the survival rate (Fig.2).

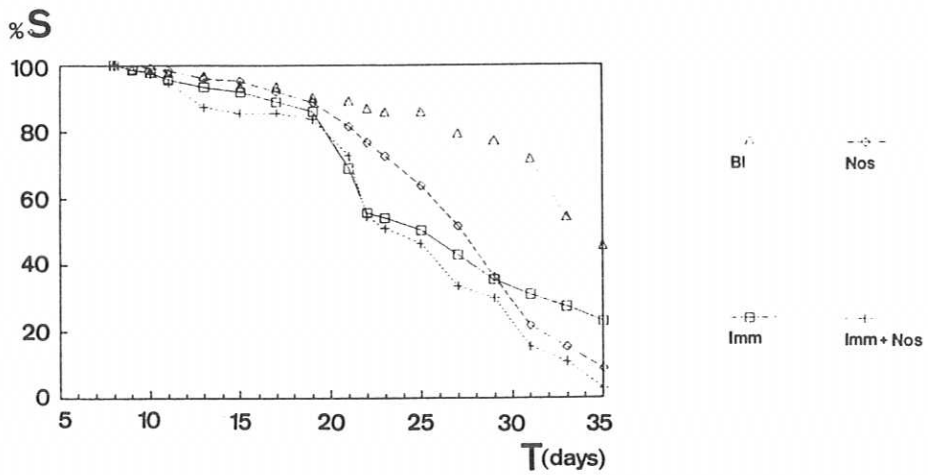

Fig.2: Survival test

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UNINFESTED PLANTS PROFIT FROM THEIR INFESTED NEIGHBOURS

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Keywords:
Herbivore, predators, tritrophic interaction, communication, plant defence, spider mites.

SUMMARY When infested by spider mites (*Tetranychus urticae*), plants produce volatile cues, toward which predatory mites (*Phytoseiulus persimilis*) are attracted. In this study we investigated whether also nearby uninfested plants might profit from this airborne information. It is shown that predatory mites show a preference for uninfested Lima bean and cotton plants that had been exposed to odours from spider mite–infested conspecifics, compared to unexposed control plants. Our experiments strongly suggest that uninfested plants are better protected against herbivory when exposed to airborne chemicals, released by their infested neighbours.

INTRODUCTION

Plants may defend themselves against herbivory by attracting natural enemies of the herbivores (Price *et al.*, 1980; Price, 1986). One way of achieving this is through release of volatile substances, in response to herbivore damage. Parasitoids (e.g. Nadel & Van Alphen, 1986; Turlings *et al.*, 1990) as well as predators (e.g. Sabelis & Van de Baan, 1983; Sabelis & Dicke, 1985; Dicke, 1988) were shown to be attracted olfactorily by plants that are infested with prey.

One tritrophic system that is studied extensively since the last eight years consists of predatory mites (*Phytoseiulus persimilis* Athias-Henriot), spider mites (*Tetranychus urticae* Koch) and various plant species. All plant species tested were shown to be attractive to *P. persimilis*, upon infestation by *T. urticae* (Dicke & Sabelis, 1988). Chemical investigations revealed that *T. urticae*–infested Lima bean plants (*Phaseolus lunatus* L.) emit a number of volatiles, that are not emitted by artificially damaged or undamaged Lima bean plants (Dicke, 1988; Dicke *et al.*, 1990). Some of these substances were tested separately in an olfactometer, and four of them attracted *P. persimilis* females, *viz.* the terpenoids linalool, (E)-ß-ocimene and 4,8-dimethyl-1,3(E),7-nonatriene, and the phenolic methyl salicylate (Dicke *et al.*, 1990).

Since the predator–luring substances are volatile, they will also reach downwind neighbouring plants. Such uninfested plants run potentially high risks of becoming infested in the near future. This raised the question whether uninfested plants can profit from the airborne information released by their infested neighbours. To obtain an answer, we tested whether such uninfested plants are also attractive to predatory mites. Additionally, we carried out a preliminary chemical analysis.

MATERIALS AND METHODS

Two parallel three-compartment wind tunnels (see figure 1) were positioned in a climate room (T= 25±1°C, RH= 70±5%, light:dark=16:8 hours). Compartments of both wind tunnels consisted of an iron frame, around which plastic foil was wrapped. Air was continuously floating through both wind tunnels, entering from the exterior of the room and led out of the building. Groups of individually potted seedlings of cotton (*G. hirsutum* var. Acala SJ-2) or Lima bean (*P. lunatus* cv. Carolina or Sieva) were placed in compartments. Two types of experiments were performed: 1. In one three-compartment wind tunnel, plants in compartments A and C (see fig. 1) were uninfested, whereas plants in compartment B were infested by ample amounts of *T. urticae*. In this set-up, plants in compartment C are exposed to odours from infested plants. The unexposed plants from compartment A serve as a control. 2. The two parallel wind tunnels were used simultaneously; compartments A1, A2 and B3 contained uninfested plants, whereas compartment B2 contained plants that were infested by *T. urticae*. Now plants in compartment B3 are exposed to odours from infested plants, and plants in compartment A3, which are exposed to uninfested conspecifics, serve as a control.

Groups of 6 to 8 plants –2 to 4 leaves per plant– were transferred from the wind tunnel compartments to the Y-tube olfactometer, to test their attractiveness to *P. persimilis*.

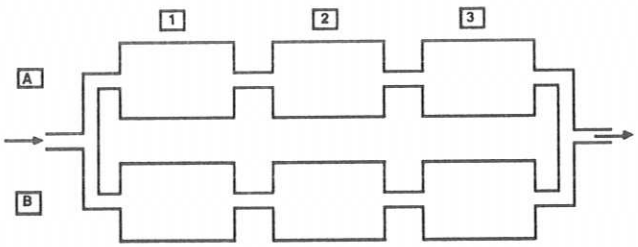

FIGURE 1: *Schematic representation of the two parallel three-compartment wind tunnels. The small arrows indicate the direction of the air stream.*

Y-tube olfactometer The olfactory response of *P. persimilis* females to uninfested plants, that had or had not been exposed to odours from spider mite–infested conspecifics, was tested in a Y-tube olfactometer (see Sabelis & Van de Baan (1983) and Bruin & Sabelis (1989) for a more extensive description of set-up and procedures). The predatory mites –from a stock-culture on *T. urticae*, on Lima bean leaves– were tested one at a time. The olfactometer was in a second climate room (T= 25±1°C, RH= 60±10%). After each trial all plants were put back in the wind tunnels.

Chemical analysis Leaves from Lima bean plants, from both compartments A3 and B3, were put in a glass jar, through which an air stream was generated. At the outlet of the jar, headspace volatiles were collected on Tenax-TA adsorbent. The volatiles were released from the adsorbent by heating in a Thermodesorption Cold Trap Unit. Combined gas chromatography and mass spectrometry revealed the identity of the compounds (see for details Dicke *et al.*, 1990).

RESULTS

Predator behaviour Females of *P. persimilis* preferred uninfested Lima bean and cotton seedlings that had been exposed to odours from spider mite–infested conspecifics, to unexposed seedlings (Table 1). Also, the predatory mites preferred uninfested cotton seedlings that had been exposed to infested ones, to uninfested plants that had been exposed to uninfested ones (Table 2). In both types of experiment, the preference is not shown initially, but only after a certain time of exposure.

TABLE 1: Response of *Phytoseiulus persimilis* females in a Y-tube olfactometer.

PLANT SPECIES	ODOUR SOURCES	DURATION OF PLANT EXPOSURE (days)	n(–)	n(+)	n(0)	n(+)/(n(+)+n(–))	p
Lima bean	A1 versus A3	3	18	14	3	0.44	0.30
		5	5	19	1	0.79	0.003
	B1 versus B3	4	14	16	4	0.43	0.86
		5	5	20	5	0.80	0.002
Cotton	A1 versus A3	2	9	9	2	0.50	0.59
		4	11	22	2	0.67	0.04
	B1 versus B3	2	9	16	5	0.64	0.11
		5	7	16	7	0.70	0.047

ODOUR SOURCES: refers to groups of uninfested plants, coming from wind tunnel compartments A1, A3, B1 or B3 (see fig. 1); n(–): number of mites that reached the far end of the arm, containing unexposed plants (from compartment A1 or B1); n(+): number of mites that reached the far end of the arm, containing plants that had been exposed to odours from infested plants; n(0): number of mites that did not reach the far end of either arm within 5 minutes; p: critical level (sign test).

TABLE 2: Response of *Phytoseiulus persimilis* females in a Y-tube olfactometer.

PLANT SPECIES	ODOUR SOURCES	DURATION OF PLANT EXPOSURE (days)	n(–)	n(+)	n(0)	n(+)/n(+)+n(–)	p
Cotton	A3 versus B3	5	7	9	6	0.56	0.40
		8	3	15	6	0.83	0.004

ODOUR SOURCES: refers to groups of uninfested plants, coming from wind tunnel compartments A3 or B3 (see fig. 1); n(-), n(+): number of mites that reached the far end of the arm, containing plants that had been exposed to odours from uninfested or infested plants respectively; n(0): number of mites that did not reach the far end of either arm within 5 minutes; p: critical level (sign test).

Chemical analysis The preliminary chemical analysis revealed that considerable shifts may occur in the headspace composition of uninfested Lima bean plants upon exposure to infested conspecifics. For example, uninfested plants that never experienced *T. urticae* infestations emitted quantities of (E)-ß-ocimene and 4,8-dimethyl-1,3(E),7-nonatriene that are much lower than or equivalent to the quantities of (Z)-3-hexen-1-yl acetate. However, plants that had been exposed to odour of spider mite–infested plants emitted quanitities of (E)-ß-ocimene or 4,8-dimethyl-1,3(E),7-nonatriene, that are equivalent to or much higher than the amounts of (Z)-3-hexen-1-yl acetate: a maximum of 26 times the amount of (Z)-3-hexen-1-yl acetate was found for (E)-ß-ocimene and 5 times for 4,8-dimethyl-1,3(E),7-nonatriene. The terpenoids (E)-ß-ocimene and 4,8-Dimethyl-1,3(E),7-nonatriene are known to attract *P. persimilis* (Dicke *et al.*, 1990).

DISCUSSION

Our experiments show that exposure to volatiles, that are released by infested plants, causes nearby uninfested conspecifics to be attractive to predators. Thus, uninfested plants can profit from their infested neighbours. However, the mechanism underlying the effects shown remains to be elucidated. We can think of two possibilities. Firstly, preference of the predatory mites for uninfested plants exposed to infested neighbours could be based on a response to volatiles which first adsorb onto waxy layers of the exposed plant's surface, and then volatilize again secondarily (*cf.* Wall *et al.*, 1981; Wall & Perry, 1983). This would leave the exposed plants passive, although not less protected.

Alternatively, the predatory mites might have responded to substances that were produced *de novo* by the exposed plants. In that case the exposed plants are actively involved in luring the predators. Active responses of uninfested plants, exposed to conspecifics that were infested by herbivores other than spider mites, have been suggested for some plant species (Baldwin & Schultz, 1983; Rhoades, 1986; but see

Fowler & Lawton, 1985; Zeringue, 1987). Recently, Takabayashi *et al.* (1990) found chemical evidence for active responses in detached uninfested Lima bean leaves, exposed to cues from spider mite–infested leaves. The results of the preliminary chemical analysis presented in this paper, can be explained both by adsorption followed by secondary release, as well as by active production.

However, whatever the underlying mechanism, exposure to volatiles emanating from infested plants results in better protection against herbivores in uninfested neighbours.

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RESPONSE OF THE PARASITOID WASP *COTESIA GLOMERATA* TO ODOUR CUES FROM THE HOST-HABITAT COMPLEX.

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Keywords:
Tritrophic interactions, infochemicals, flight tunnel, *Pieris brassicae*.

Summary

Responses of the parasitoid *Cotesia glomerata* (L.) (Hym., Braconidae) to different host and host plant related odours were investigated in a flight tunnel. Dual choice tests suggested that cabbage plants damaged mechanically or infested by larval *Pieris brassicae* (L.) (Lep., Pieridae) are a major source of volatiles attracting the wasps to the host habitat. Feces and host larvae, two major components of a complete plant-host complex, are less attractive. The percentage of wasps responding in the bioassay averaged below 20%. Responsiveness of the 1-year-old laboratory strain was therefore compared to that of field collected wasps. The response of the latter was higher and less variable than that of the lab strain.

INTRODUCTION

Cotesia glomerata is a cosmopolitan, gregarious larval endoparasitoid of various Lepidoptera, most of which do feed on cruciferous host plants. The large cabbage white *Pieris brassicae* is one of its common hosts in Europe and Asia, for which high rates of parasitism have been reported (Laing & Levin, 1982; Rataul, 1976). Successful parasitism of the host is a process that is of vital importance for parasitoid reproduction and fitness. This process can be divided into a series of behavioural steps beginning with host habitat location (Vinson, 1976). Various studies have shown that insect parasitoids, while foraging for hosts, use a variety of volatile chemical cues, or infochemicals (Dicke & Sabelis, 1989), associated with their hosts (kairomones) and/or host plants (synomones).

The aim of the present study was to investigate the importance of olfactory stimuli in the host-habitat location behaviour of *C. glomerata*. This research is part of a program studying tritrophic interactions between this parasitoid (and related species) and its hosts and host plants in order to gain insight into the evolutionary strategies and mechanisms by which plants defend themselves directly and indirectly to insect attack.

The process of host habitat location by *C.glomerata* seems to be primarily based on odour discrimination during flight and less on visual stimuli (Kitano, 1978). Sato (1987) suggested that one of the first cues in the chemically mediated orientation behaviour of the wasps is not associated with the hosts themselves, but with the host plant or an interaction between these two trophic levels. In order to investigate this

question, and to develop a bioassay for future research, we used a flight tunnel to determine which volatile components from the host-habitat complex are attractive to the wasps.

MATERIAL AND METHODS

C. glomerata females were obtained from a culture that was originally collected in the vicinity of our laboratory and maintained for about 15 generations on laboratory cultured larvae of *P. brassicae*. The host culture was reared on Brussels sprouts (*Brassica oleracea* cv. Titurel). Parasitoid cocoons of uniform age were placed in a nylon-gauze emergence cage (35x30x40 cm) for random mating. Two to five days after emergence test females were isolated and given an oviposition experience with a first or second instar *P. brassicae* on a Brussels sprouts leaf. Hereafter they were kept individually in plastic petri dishes (ϕ 5cm) with a droplet of honey at ca. 15 °C and 12 h photophase. Test females were used first within 1-2 days after isolation.

A schematic view of the flight tunnel used throughout this study is shown in Fig. 1. The tunnel, 100 cm long and 50x50 cm cross-section, is partly made of plexi glass. A detailed technical description of the set-up is given by Noldus (1989). Observations (direct or with the aid of video-equipment) took place at 20 °C, 60-70% rh and ca. 2000 lux inside the tunnel. The speed of active-coal filtered air pulled through the tunnel averaged 13.7 (± 4.7) cm/sec.

Test odours were released from two odour sources in separate glass-tubes (ϕ 5 cm, length 10 cm), placed horizontally in a stand 13.5 cm above the tunnel floor. The downwind openings of these odour-source tubes were covered with nylon gauze (mesh-width 2.0 mm). Wasps were released 37.5 cm downwind from the odour tube opening by placing the containment petri dish on the bottom of the tunnel. A smoke test showed that the release site was well within the odour plume. The lid of the petri dish could be raised from outside the tunnel by a string to avoid disturbance of the test wasp. Each test was terminated either immediately after a wasp had landed, or 10 min. after wasp release if no odour-source orientated flight had been made.

In a first series of experiments the response of wasps was tested to various components of the plant-host complex in eight dual choice combinations of odour sources. Odour sources tested were undamaged clean leaves, infested leaves including larvae of *P. brassicae*, their feces, silk and feeding damage or one of the latter sources separately. Mechanical damage was obtained by a transverse cut in clean leaves. Leaves were cut from 8-10 weeks old *Brassica oleracea* cv. Titurel plants. The stems were wrapped in wet cotton wool and aluminum foil to prevent desiccation. For development of plant reaction to feeding damage, larvae were placed on the leaves one day prior to testing. In these experiments the response to a certain pair of odour sources was tested from 1 up to 9 times per female with time intervals of 1/2 to 2 days. The number of wasps tested per comparison varied between 20 and 115.

The percentage of wasps responding in the test by an oriented flight to one of the odour sources within 10 min. after release was not higher than 20 % throughout the first series of experiments. It was then decided to compare the response of the laboratory strain with that of field collected *C. glomerata*. Wasps were collected near our laboratory by exposing laboratory reared, young *P. brassicae* larvae on Brussels sprouts plants to parasitism outdoors for a couple of days. Exposed hosts were reared in the laboratory similar to the standard laboratory culture. After emergence, "field wasps" were conditioned for experiments as the "laboratory wasps". In the flight tunnel these two kinds of wasps were given a choice between clean leaves and infested leaves (with first instar *P. brassicae*, frass and silk) as an odour source only.

In this second experiment each female was tested in a first trial for a maximum duration 5 minutes. Those wasps that carried out an oriented flight (landing on one of the odour source tubes) as well as those that did not fly away from the release site were given a single trial only. By contrast, wasps that had carried out an unoriented flight (landing on one of the sides, top or bottom of the tunnel) were placed back in the petri dish on the release site. Thus the latter were given up to four more flight trials. This experiment was conducted twice, in June and in September, with 40 wasps of each strain tested per experiment.

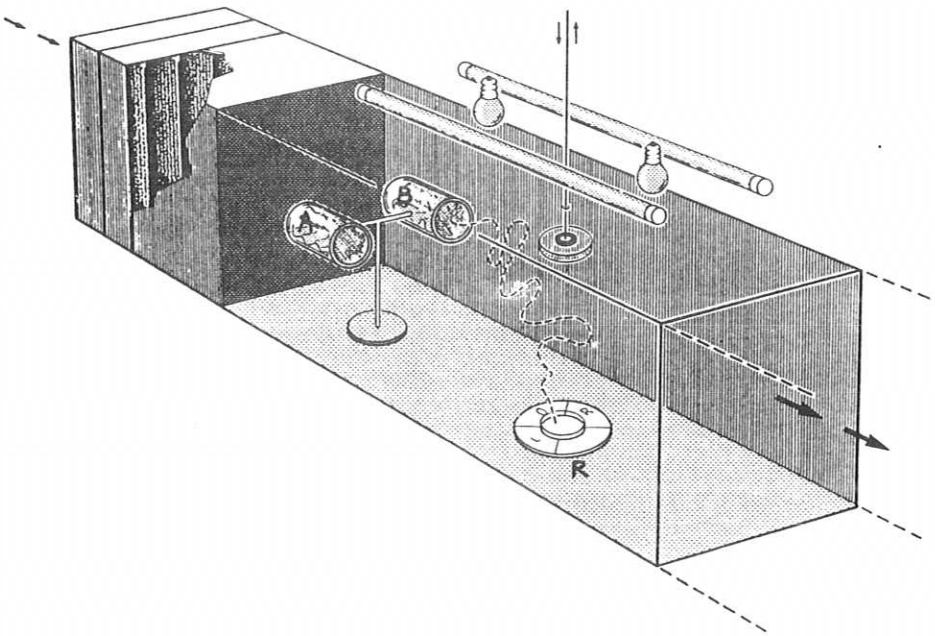

Figure 1. Flight tunnel for bio-assaying the response of Cotesia glomerata to volatiles from different host or host habitat related odour sources offered in a dual choice. R = release site; A, B = odour sources.

RESULTS

- Behaviour description

The flight behaviour differed between wasps. Some flew away suddenly, whereas others showed characteristic pre-flight behaviour: pointing of the antennae, vibrating the wings and positioning for take-off. Pre-flight behaviour did not provide, however, an unambiguous clue whether a wasp would carry out an odour-orientated flight or not. An example of an oriented flight is shown in Fig. 1. The path is not straight but characterized by frequent loops and zig-zagging, especially prior to landing on the odour-source tube. The choice between the two odour sources appears to be made at an early stage of the flight.

- Odour choices

For each test wasp the median odour choice was determined from the series of replicates per wasp. The overall response of wasps flying at least once to one of the odour sources was only 19.4% and differred greatly between odour combinations. Results for the different combinations are summarized, in terms of the preferred choice made by the wasps, in Table 1. Initial tests showed that wasps did not respond to clean air or clean leaves. Oriented flights were obtained, however, for all other tested odour sources. Wasps showed a significant preference for complete infested leaves over clean leaves. Feces was significantly more attractive than larvae, but less attractive than the odour released from feeding damage. The response to mechanical damage and feeding damage was not different, whereas mechanically damaged leaves were preferred over nonfeeding larvae.

Table 1. Overview of the odour choices of *Cotesia glomerata* to different odours from the host-habitat complex.

Odour source A		Odour source B
complete leaf	>	clean leaf
feces	>	larvae
feeding damage	>	feces
feeding damage	≥	mechanical damage
mechanical damage	>	dead larvae

- Lab vs field strain

Females of both the lab and the field strain showed significantly more oriented flights to infested than to uninfested leaves (Sign test, $p<0.001$). Only one female flew to the uninfested leaves (Fig. 2). The field strain showed a high response in both the June and the September trials (78 and 82% of the females responding, respectively). Its response was less variable than that of the lab strain (27% responding females in June and 73% in September). For both trials, the response of the lab strain is significantly lower than than of the field strain (X^2-test; $p<0.01$). In June the lab strain showed, beside many nonresponding females, a high percentage of unoriented flights among responding females.

Oriented flights to infested leaves generally took place within one or two trials (Fig. 3). The distribution of the number of trials and wasp responsiveness is significantly different between the lab strain and the field strain in both trials (Wilcoxon sample test; $p<0.01$). Few females of the lab strain in June had an oriented flight to infested leaves in the first trial. In September, however, relatively many females of the lab strain did have an oriented flight to infested leaves in their first trial.

DISCUSSION

The results of the odour-source combinations investigated in the first experiment show that the highest response was obtained with a complete, infested leaf. The lowest response was obtained for a clean, uninfested leaf and the responses to the other odour sources were intermediate to these extremes. These results suggest that the olfactory stimuli deriving from the ongoing interaction between host plant and host (the first and second trophic level) are of primary importance in the host-habitat location behaviour of the wasps. Damaged plants from which larvae have been removed, or plant materials which have been digested by the host (feces) are

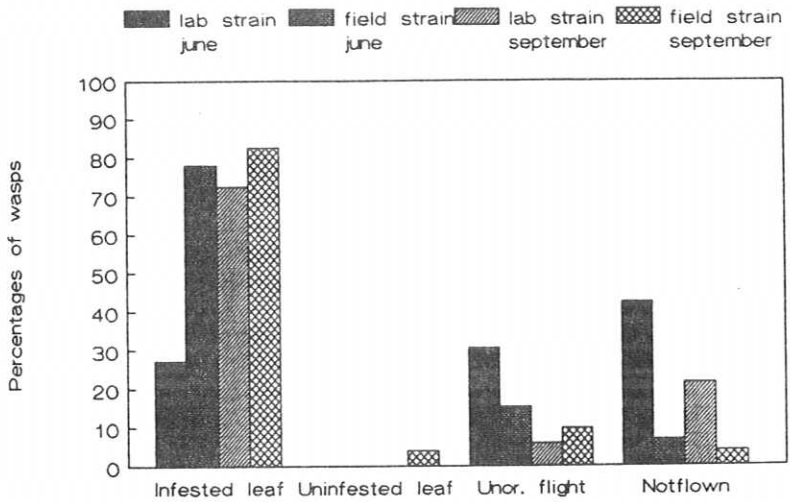

Figure 2. Distribution of flight responses to infested and uninfested cabbage leaves for lab and field strain of Cotesia glomerata tested in June and in September.

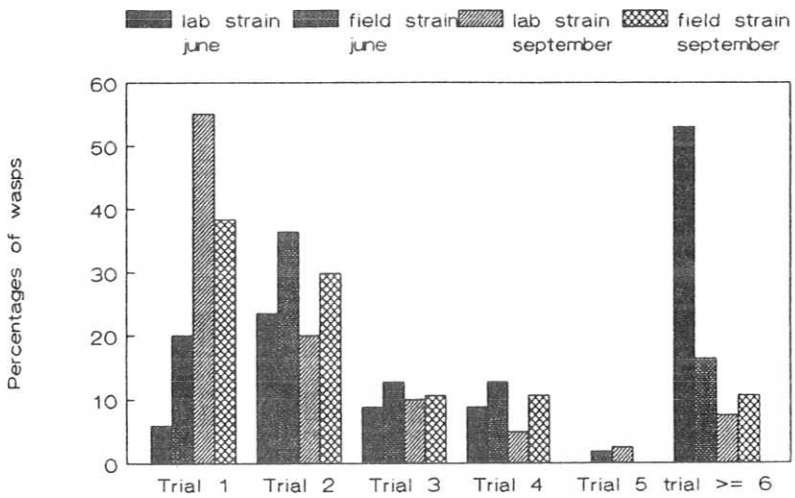

Figure 3. Distribution of the number of trials necessary for an oriented flight to infested cabbage leaves by Cotesia glomerata from a lab and field strain tested twice in a flight tunnel.

"leftovers" of a previous interaction between the first two trophic levels and seem to be a secondary source of infochemicals, being less attractive to the wasps than plant leaves on which larvae are feeding. Separately, the first two trophic levels, the (undamged) host plant and the larvae, are by themselves hardly attractive to *C. glomerata* females.

Turlings et al. (1990) reported a similar kind of odour ranking for the related generalist parasitoid *Cotesia marginiventris* (Cresson). Both *C. glomerata* and *C. marginiventris* mainly react to the odour of damaged host-plant leaves in chemically mediated orientation behaviour. Feeding larvae signal the presence of potential hosts. The response to a preferred component or a mixture of components produced and/or released as a result of the host's feeding activity can be easily understood as a profitable element in the host searching strategy of the wasps. In addition, a function of this response within a tritrophic context as a mechanism helping the defense of the plant to insect attack is also suggested, since the response is not elicited to such a great extent by the host larvae themselves.

The response of field collected wasps initially seemed to be much higher than that of laboratory reared *C. glomerata*, indicating a possible genetic deterioration of the lab strain. In the second comparison however, which was concurrent with a breeding experiment between field and lab wasps, the laboratory strain showed an increased response. Both experiments confirmed the conclusion from the first series of experiments: stimuli deriving from the interaction between host plant and host are more important in the host finding process than stimuli deriving from the host plant alone. However, inbreeding therefore can no longer be considered as a plausible explanation for the low response of the lab strain, although its response was still significantly lower than that of the field strain. After the two current tests, the response of field collected wasps seemed to be more constant than that of lab cultured wasps. When the research continued, however, it turned out that also field wasps can show, at some times, a low response. Variability in response thus seems to be caused by certain unknown experimental conditions, such as rearing and conditioning procedures of the wasps, which will be investigated further.

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DISTRIBUTION IN SPACE AND TIME OF *TRIALEURODES VAPORARIORUM* (WESTWOOD) ON *GERBERA*: DOES HOST PLANT ARCHITECTURE INFLUENCE THE DISPERSAL AND DISTRIBUTION OF THE WHITEFLY?

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Keywords:
Trialeurodes vaporariorum, ornamentals, dispersal, distribution, plant architecture.

1. Introduction

The relationships between the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) and its parasitoid *Encarsia formosa* Gahan have been studied intensively on vegetable host plants (e.g. Noldus & van Lenteren, 1990). However *T. vaporariorum* is not only a very important pest insect in vegetable crops but in ornamentals too. Population dynamic studies have been done lately on the relation between whiteflies and the ornamental plant Gerbera (Dorsman & van de Vrie, 1987). As a result, we know how to place Gerbera in a series of host plants concerning their quality for the pest insect. Gerbera is not a very good host plant for *T. vaporariorum* and is in this respect comparable to tomato (Dorsman & van de Vrie, 1987). Therefore one would not expect a problem for the parasitoid *E. formosa* to control whitefly on Gerbera.
In preliminary trials in commercial glasshouses, however, there have been problems with biological control. The exact cause was not easy to identify. This led to the decision to do more detailed research in this field.

1.1. Leaf hairs, host plant architecture and biological control. One of the possible obstacles in the biological control of whiteflies in a Gerbera crop might be the leaf structure, particularly the leaf hair density, -length and -form. The trichomes can hamper the wasp in walking on the leaf through their large density, their length or their curly structure. Bearing in mind that *E. formosa* has a random walking pattern (van Lenteren et al., 1976) on leaves and that the capacity to encounter hosts is therefore depending for a large degree on the walking speed, one can imagine the importance of the leaf structure. This has been shown previously for cucumber (van Lenteren & de Ponti, 1990). On the other hand the leaf hair density might influence the pest insect as wel. In cotton it was found that the hair density is positively correlated with the population size of the cotton whitefly *Bemisia tabaci* (Butter & Vir, 1989). Such a positive effect on *T. vaporariorum* populations in 'hairy' Gerbera varieties could hamper the biological control.
Another unknown factor is the host plant architecture. Compared to the vertically grown vegetable crops, Gerbera is more horizontally oriented: it has a rosette shape. How does *T. vaporariorum* react to this type of architecture? Is its dispersal influenced? Does this lead to another distribution on the host plant Gerbera than on a vegetable plant as e.g.

tomato? And how does this architecture affect *E. formosa*?
In this paper we will deal with the pest insect only. Its distribution and dispersal on a host plant with a low hair density (variety Terra Fame, refered to as Fame), will be discribed. The same experiments have been done on a variety with a high hair density (Parade): the results were similar.

2. Distribution and dispersal of *T. vaporariorum* on tomato

The within-plant movement of whiteflies was studied by Noldus et al. (1985) on the host plant tomato. They found a concentration of emergence early in the morning and no emergence or movement when it was dark. The adults start to leave the place of emergence after eight hours. The dispersal activity was highest in the afternoon between 15.00h and 17.00h. The whiteflies concentrated in the upper leaf layers (2.5 leaves from the top). After two and a half days dispersal had more or less stopped.

3. Distribution of *T. vaporariorum* on Gerbera

3.1. Material and method. The experiments were conducted in a small glasshouse on ten Gerbera plants of uniform age and size with 50 adult whiteflies per plant. The plant leaves were classified in three groups: old, medium and young leaves. The classification was made on a morphological base, as well as on the different leaf surface of the three groups. Exact details are given in Sütterlin et al. (1990).
3.2. Results and conclusion. During the whole experimental period we recovered 37% of the adults on the 'old' leaves, 49% on the 'medium' leaves and 14% on the 'young' leaves. On the basis of the total leaf surface per age class and on the premise that whiteflies distribute at random, we expected the following distribution: 28%, 66% and 6% (Sütterlin et al., 1990). The observed and expected distribution differ significantly. The adults stay on the younger leaves more often than on old or medium leaves. A second parameter indicating a 'preference' of the whiteflies for young leaves is the number of eggs laid per female per 24h.
Table and figure 1 show that many more eggs are laid per female on young leaves than on old or medium leaves.

Table 1: The number of eggs per female per 24 hours on three leaf types of Gerbera, variety Fame (500 females tested).

old	medium	young
0.7	1.5	2.6

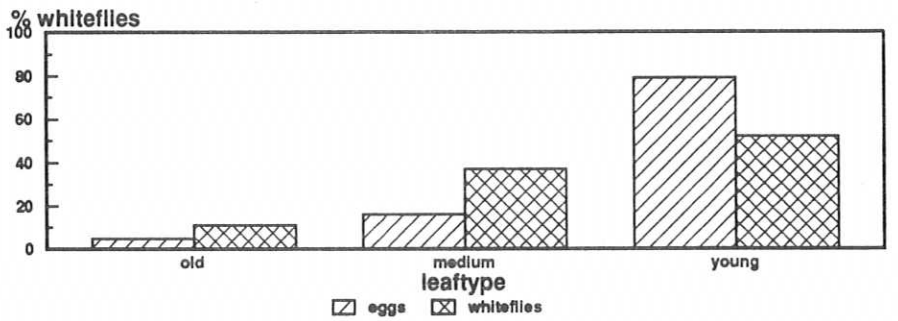

Figure 1: The distribution of whitefly eggs and adults among three leaf types of Gerbera, variety Fame.

4. Dispersal of *T. vaporariorum* on Gerbera

4.1. Material and method. Five uniform Gerbera plants were placed on a table. Each plant had ten leaves. A piece of a Gerbera leaf (ca. 1.5cm x 2.5cm) full of not yet emerged whitefly pupae was pinned on the underside of the leaf on which whiteflies would normally emerge (leaf number five from the top) in every plant (fig. 2). From early morning onwards, the emergence of the pupae and the movement of the adults was observed. After the whiteflies had emerged (10.00 to 11.00 a.m.) the further movement of the adults was followed every hour until dark. Then all adults which had remained on the 'leaf of emergence' were caught. During the second day the observations took place on the same plants up to 10.00 p.m. The temperature was kept at about 23 C, the relative humidity was 65% and the photophase 16h.

4.2. Results and conclusion. Emergence began after lights went on at 6.00 a.m. The emergence peak occurred between 9.00 and 10.00 in the morning, which agrees with results of whitefly emergence on tomato (Noldus et al., 1985). Some four hours after the first emergences, movement away from that leaf began and five hours after the emergence peak, a strong migration to the youngest Gerbera leaves was seen. The distribution became stable about nine hours after the emergence top (fig. 3). A similar trend in dispersal was found in Noldus et al. (1985). In our experiments the migration process went much quicker in all aspects. The choice for the young leaf type was made in a very early stage of the proces. The movement by the adults could be imagined as one directed towards the middle of a helix with a certain upwards movement. This vertical component in the host plant architecture might help the adults to find their way to the youngest leaves. Next to this architecture component a visual stimulus (e.g. colour of the leaves) could be a help to whiteflies in judging the leaves from a distance, before landing. Whiteflies can react to reflection of light in a certain spectrum (van Lenteren & Noldus, 1990).

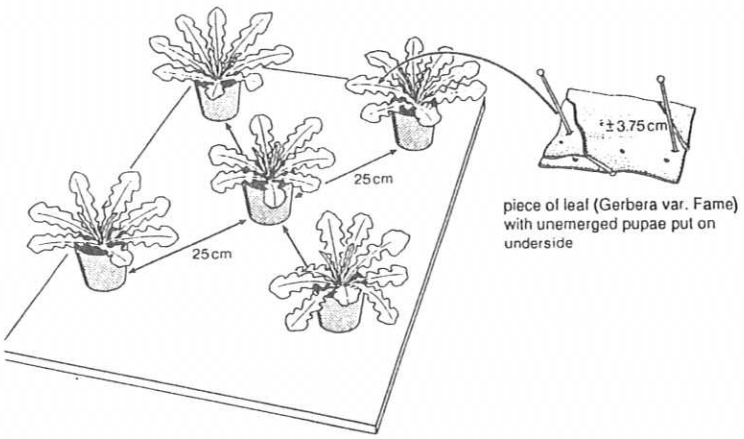

Figure 2: Set-up of the dispersal experiment.

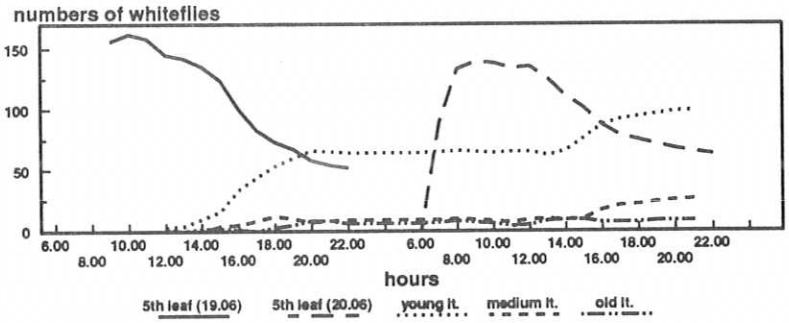

Figure 3: The dispersal of newly emerged whiteflies in a Gerbera plant, variety Fame, shown during 40 hours. Observation on 19.06.1990 and 20.06.1990.

5. Leaf choice experiment

The position of a leaf in a plant might influence leaf choice by whiteflies. In Gerbera, the most central leaves of the rosette are oriented above medium or old leaves. To judge the potential effect of this vertical component on the leaf choice of whitefly females we designed an experiment with the different leaf types offered at one level to the insects.

5.1. Material and method. In a black cage three glass vials with three leaves of the different leaf types were placed in a semicircle around a release point (fig. 4). The position of each leaf type was rotated to correct for site effects. The experiments were done each with 25 females and three times replicated. We observed the first flight of the females from their release point to one of the three leaves. Thus we obtained the females 'first choice' for landing on a certain leaf. Afterwards the distribution of the whiteflies

was traced every hour during one day. Temperature was kept on about 21 C, the relative humidity was 60% and the photophase 16h.

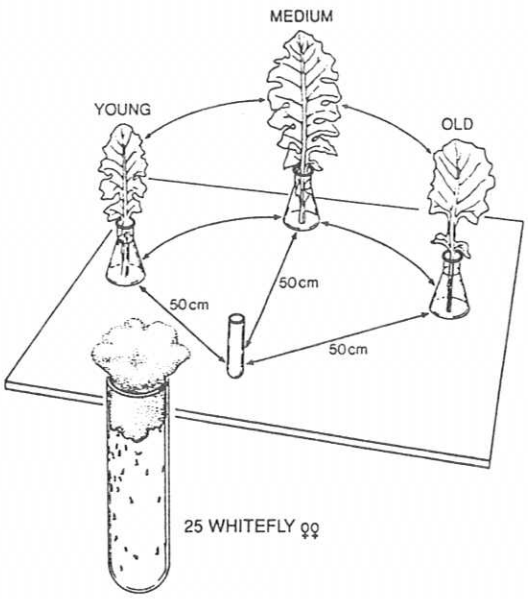

Figure 4: Set-up of the leaf choice experiment.

5.2. Results and conclusion. The expected percentage of landings, according to the leaf surface and based on the assumption of no preference, was 24% on the old leaves, 49% on the medium leaves and 27% on the young leaves. The 'first choice' landings of the females was not significantly different from the expected distribution. The distribution of whiteflies after 24 hours, however, was significantly different (table 2) from both the expected and initial distribution (chi-square: 44.72 and 33.84, both $p << 0.001$). A larger number of whiteflies did finally choose for the young leaf type. They leave the old and medium leaves. Based on these results we hypothesize the leaf selection behaviour of whiteflies in a Gerbera crop to be as follows. Whiteflies which have recently emerged will randomly land on leaves of different ages (different qualities), they will stay on good leaves (in this case the young leaves) and move away from lower quality leaves (medium and old). After some time most whiteflies will have arrived at the younger leaves.

6. Final conclusions

In the experiments with intact Gerbera plants, whitefly adults were not distributed evenly among the leaves. In contrast many more were found on the younger leaves. The dispersal process itself began several hours after emergence. The whole process lead to a rather quick aggregation on the younger leaves. The distribution became stable nine hours after the dispersal process started. When eliminating the vertical component in the host plant architecture, the distribution was first at random on the three leaf types, but

later the distribution had changed, the whiteflies again concentrated on the youngest leaves. There, many more eggs are laid per female than on the medium or old leaves.

Table 2: The expected and observed percentage whiteflies on three leaf types of Gerbera, variety Fame. Leaves were offered on one level.

leaf type	% expected	% at first choice	% at end situation
young	27	31	56.5
medium	49	40	33.5
old	24	29	10.5

Acknowledgements

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A STUDY OF HOST PLANT ADAPTATION IN THE GLASSHOUSE WHITEFLY (*TRIALEURODES VAPORARIRORUM* WESTWOOD)

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Keywords:
Trialeurodes vaporariorum, host plant adaptation, life history studies, genotype-environment interaction.

INTRODUCTION

Our project contains three major aspects:

1 Conduction of long term experiments over several consecutive generations to comparatively assess the adaptation of whitefly to several different host plants (mostly of commercial horticultural importance).

2 Conduction of experiments to assess the influence of *Encarsia formosa* (natural enemy of whitefly) upon host plant adaptation by the whitefly.

3 An analysis of information obtained from glasshouse advisors as to the occurrence of host plant adaptation in commercial glasshouses and the influence of whitefly migration into glasshouses upon this adaptation process.

The inter-relationship of these aspects are summarised in fig. 1.

METHOD

The general methodology used is based upon ideas from Gould (1979), Stearns (1977) and Via (1984,1989,1990). In the experimental set up various life history parameters are measured / calculated per host plant per generation. The host plants currently under study are tomato (*Lycopersicon esculentum* and *Lycopersicon hirsutum*), gerbera (*Gerbera jamesonii* (two varieties thereof)), sweet pepper (*Capsicum annum*), chrysanthenum (*Chrysanthenum hortorum*) and cucumber (*Cucumis sativus* (4 varieties thereof)). The whitefly (*Trialeurodes vaporariorum* Westwood) population used is a population reared on *L. esculentum* var. moneymaker for at least 20 years.

RESULTS AND DISCUSSION

Our own studies to date indicate the following:

* the deterrent properties of a host plant are of key importance in delaying the development of resistance to it at the antibiosis level.

* the distribution of mortality between larval stages of an adapting species offers the most incisive and accurate insight as to the rate at which a species is adapting to a given host plant.

* the genetic history of an adapting population with regards to previous host plants that it has developed upon, severely determines which new host plants it can adapt to and the extent and rate at which such an adaptation can occur.

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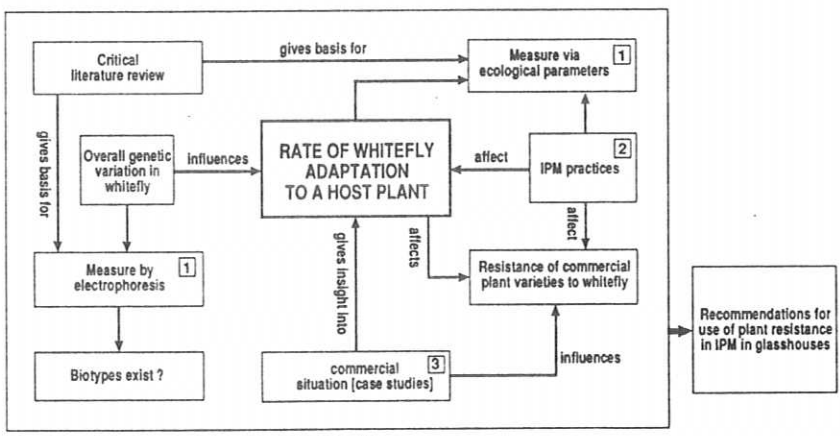
fig 1. A diagram indicating how the various facets of the research program interrelate with each other.



note numbers as [1] refer to points in the section "RESEARCH AIMS"

INTRASPECIFIC COMPETITION IN THE CARPENTER BEE *XYLOCOPA PUBESCENS* AND ITS IMPLICATIONS FOR THE EVOLUTION OF SOCIALITY

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Keywords:
Xylocopa, evolution of sociality, resource competition.

Summary

In the facultatively social species *Xylocopa pubescens* net reproductive success in general is higher in solitary nests than in social nests. Under unfavourable conditions, however, during which competition for pollen or nesting substrate is severe, net reproductive success is higher for socially nesting females. This is mainly caused by a decrease in net reproduction in solitary nests as a result of high brood mortality through pollen robbery. Shortage of pollen and of nesting substrate can enhance the occurrence of pollen robbery in the population. Therefore, intraspecific resource competition can be of importance in the profitability (in terms of net reproductive success) and thus in the evolution of sociality.

INTRODUCTION

A major selective factor favouring the evolution of a primitive form of sociality in the Hymenoptera is generally thought to be parasite and predator pressure (Lin & Michener, 1972; Andersson, 1984). In socially breeding birds and mammals, intraspecific competition is considered to be of more importance (*e.g.* Emlen, 1978; Koenig & Pitelka, 1981). In this paper I will try to show the impact of ecological factors on the reproductive success of solitary and socially nesting females of *Xylocopa pubescens* .

Xylocopa pubescens is a facultatively social carpenter bee, which makes elaborate three-dimensional nests in soft or decaying wood. After hibernation in a cluster of 5-10 individuals (males and females together), all bees except one female disappear from the nest during early spring. The remaining female starts a phase of solitary reproduction. (Gerling et al., 1981). After a month the first young males and females emerge. These teneral young are fed nectar trophallactically by their mother, and they eat pollen from the pollen slant (Velthuis & Gerling, 1983, and Van der Blom & Velthuis, 1988). Approximately two weeks after emergence the young females may either leave the nest in order to start a nest of their own, or they may stay in their parental nest and take over the reproductive dominance from their mother (or, occasionally, from a sister). During a take-over the young female may open one or more of the newly made cells, thereby destroying its contents. If the take-over is successful, the mother (or sister) may either leave the nest and try to found or take over another nest, or she may stay as a guard bee.

Thus, we see three phases of colony development:

- a solitary phase
- a 'subsocial' phase (mother with tenerals)
- a social phase, in which a nest contains at least a guard bee and a forager. The latter is the sole egg layer in the nest.

During all of these three phases intruders may come to take over the reproductive dominance. In contrast to a take-over by a nestmate, fierce fights usually occur, often lasting several hours. Almost always part or all of the brood present is destroyed. As might be expected, guarded nests are better protected against take-overs by intruders than solitary nests (Van der Blom & Velthuis, 1988).

Moreover, during periods of pollen shortage, intruders may enter the nest in order to rob pollen, either from the pollen slant or from newly made cells (Velthuis, 1987). Pollen robbers are easy to recognize by their behaviour: they approach nest entrances cautiously, and are easily deterred if one or more of the residents are inside. Thus, social nests are continuously protected against pollen robbery, whereas solitary nests are not.

During periods of shortage of pollen or nesting sites solitary bees might be motivated to shorten the time spent away from the nest, in order to minimize the risk of being robbed or of having their nests being usurped. If this is the case, solitary females have two options: they can either cut down on reproduction, or they may start to rob pollen. The latter way of foraging takes less time than collecting pollen from flowers.

In the area where this study was conducted, nesting material is difficult to find if not provided by the investigators, and pollen availability varies tremendously within and between the reproductive seasons. This led to the hypotheses that during times of severe competition for either pollen or nesting space, (1) solitary bees suffer more from pollen robbery than socially nesting females, (2) solitary bees spend less time away from the nest and (3) try to rob pollen more often than socially nesting females, and that, (4) comparing to solitary nesting, nesting socially is more profitable under harsh conditions.

MATERIALS AND METHODS

The bees were studied at the Hazeva Field Study Centre in the Negev desert in Israel, 30 km south of the Dead Sea. Nesting behaviour was studied from February until mid August 1988 (41 nests) and from May until mid August 1989 (27 nests). All data were collected from nests in boards of balsa wood, which were provided by the investigators. The bees dug their own nests, and were foraging outside.

Developmental stages of the nests were checked every other day during the evening by means of X-ray radioscopy (Gerling et al., 1981). In order to be able to recognize the bees individually, thin pieces of lead were glued onto their thoraces (for recognition in X-ray studies), which were painted with a colour code for recognition in the field.

Flight activity was checked regularly (approximately once a week) during morning hours, and specifically during 6 days at the end of May- beginning of June 1989.

RESULTS

Competition for nesting sites is very difficult to measure in a direct way, but it is reflected in the number of *X. pubescens* searching for nests, which is, in turn, correlated with the number of young females leaving the parental nest to search for a nest of their own (Fig. 1). At the end of May 1989, during a period in which competition for nesting sites seemed to be severe, flight activity of 9 solitary and 9 socially nesting females (reproductive dominants only), was recorded simultaneously (Table 1).

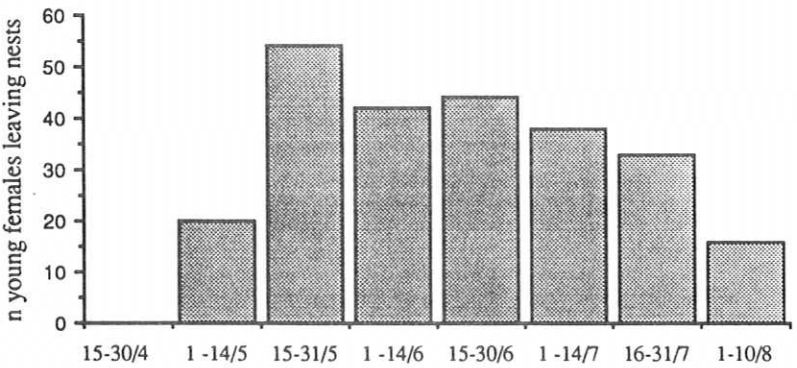

Fig.1. Number of young adult females leaving their parental nest, during periods of 15 days, April - mid August 1989

Table 1. Fouraging trips by reproductive females in solitary and social nests during 6 days between May 27 and June 4, 1989.
(Mann-Whitney U-test were performed using the averages per female)

	solitary	social		
n nests	9	9		
n flights	189	306	Chi-square	= 27.65 ***
flights/female/day	3.5	5.6	Mann-Whitney U	= 63 *
duration/flight (min)	7.34	13.32	Mann-Whitney U	= 126 ***
n flights with pollen	101	254	Chi-square	= 49.02 ***
n eggs laid	13	29	Chi-square	= 6.09 **

The socially nesting females undertook more flights per day, they took more time for each flight, and they returned to their nests more often with pollen than the solitary females. This resulted in more than twice as many eggs laid in social nests during this period of 9 days. During the same period, solitary females tried to rob pollen significantly more often (Table 2).

Table 2. Number of attempts to rob pollen by 9 solitary and 9 social females. during 6 days between May 27 and June 4, 1989.
Between brackets: the number of females making at least one attempt.

	solitary	social
attempts to rob	59 (8)	4 (1)
n flights	130	302

Chi-square = 94.01 ***

The results seem to indicate that socially nesting females in general do better in terms of reproductive success than solitary females. This is not the case. Over the whole season (1988) the net reproductive success per day of solitary nests was almost twice as high as that of socially nesting females (Table 3). This was mainly the result of the high brood

mortality in social nests, due to the within-nest competition for reproductive dominance (Table 4). During such periods of competition, which may last 1-10 days, nestmates may open each other's newly made cells.

Table 3. Reproduction and brood mortality in 41 nests in boards (1988), during their solitary and social phases (no young present).

	solitary	social	
n phases	72	48	
n days/phase	23.01	15.98	Mann-Whitney U-test, t = 2.70 **
n eggs laid	272	103	
brood mortality	48	45	
in %	17.7%	43.7%	Chi-square = 27.17 ***
net reprod./day	0.135	0.076	Mann-Whitney U-test, t = 3.52 ***

In July and August 1988 brood production became very difficult, due to a lack of flowering plants, and high temperatures allowing flight activity only during early morning. In addition, there were many bees searching for nests. Many solitary females did not breed at all. Those who did, experienced the same brood mortality as socially nesting females, although the causes of this mortality differed (Table 5). The increase in brood mortality was mainly the result of an increase in pollen robbery.

Table 4. Causes of brood mortality in 41 nests in board, 1988.

	solitary	social
total %	17.7%	45.2%
% caused by		
pollen robbery	5.9	5.1
nest take over	4.0	30.5
reasons unknown	7.7	9.6

DISCUSSION

The results show clearly that brood mortality in solitary nests is of a different nature than in social nests. In the former, the two most important reasons for brood mortality are cells being opened by intruders during usurpation, and by pollen robbers. In the latter, cells are mainly opened during competition for reproductive dominance.

Pollen robbery was nearly only performed by solitary females. From this I conclude that there is a cost to robbing, which lies in the fact that a robber might be caught in the act by the resident, returning from her foraging trip. I have seen this happen several times. During the ensuing fight, the robber is trapped inside the nest.

Table 5. Brood mortality in solitary and social nests in board during periods differing with respect to ecological constraints.

	15-Mar - 30-Jun		1-Jul - 14-Aug	
	solitary	social	solitary	social
n brood cells made	231	95	41	82
brood mortality	13.9%	50.5%	39%	39%
% caused by				
pollen robbery	3.4	7.4	19.5	2.4
nest take over	3.1	34.7	9.8	25.6
reason unknown	7.4	8.4	9.8	11

Net reproductive success of socially reproducing females in general is not higher than that of solitary females. However, in periods that competition for pollen or nesting space is
severe, reproductive dominants in social nests have greater reproductive success, even though brood mortality in these nests remains high.

It can be concluded from the difference in flight duration and activity between solitary and social nests that either social reproductive dominants are aware that their nest is guarded, and that they can stay away longer, or that solitary females know that they run the risk of losing their nests or (part of) its contents if they leave their nest unguarded for a longer period of time.

One of the first steps in the evolution of sociality in Hymenoptera is thought to be the development of a tolerance towards nestmates, and especially towards guard bees. It is generally supposed that parasitism and predation are the selective pressures involved in this first step. I have shown that, under circumstances of severe intraspecific competition in bees, a reproductive dominant can profit from tolerating a guard bee in the nest, and thus that environmental constraints can be one of the selective pressures for the evolution of such a tolerance.

ACKNOWLEDGEMENTS

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THE USE OF BUMBLEBEE COLONIES (*BOMBUS TERRESTRIS* L.) FOR POLLINATION OF GLASSHOUSE TOMATOES

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Keywords:
Bombus terrestris L., rearing, management of bumblebee colonies.

Summary
It is now known that bumblebees can pollinate tomato flowers in glasshouses during the whole growing season (January-September). This means that there is a huge market for bumblebee colonies which should be available yearround. A method has been developed for rearing bumblebee colonies continuously. The species used is Bombus terrestris. A management system for bumblebee colonies in a glasshouse is described.

INTRODUCTION

Rearing bumblebee colonies is labour intensive and therefore expensive. In respect of using bumblebees for pollination, the bumblebees have to compete with other insects, such as honeybees. The major problems when using bumblebees for pollination purposes were their price and the life cycle with a winter diapause. It was thought that rearing bumblebee colonies had less economic interest (Röseler 1979). Recently new possibilities have arisen to use bumblebees for the pollination of glasshouse tomatoes. Glasshouse tomatoes are an important crop in the Netherlands and pollination was done manually. It takes 40 hours per ha per week to pollinate tomato flowers properly. With 1500 ha of glasshouse tomatoes in the Netherlands only, and yearly labour costs of hand pollination exceeding 40 million Dfl, it becomes evident that there is a huge market for bumblebee colonies, that should be available yearround.
The Ambrosiushoeve started in 1988 to realise a yearround rearing of bumblebee colonies.

MATERIAL AND METHODS

The species used is Bombus terrestris. In order to start breeding, each queen is put into a small cage together with 3 or 4 newly emerged worker honeybees to assist her (Ptacek 1985). Sucrose solution and a little ball of pollen patty is fed. The cages are kept in the dark in a climate room at 29 ^{o}C and a relative humidity of 50%-60% Red light is used for inspection (bumblebees are red-blind). As soon as the first worker bumblebees emerge, breeding queens as well as the brood and the young bumblebees are moved to nestboxes. As soon as 80 workers have emerged, these colonies can be used for pollination.
For queen and male production, colonies are kept in the climate room.

Young queens are put in a flight cage in daylight, together with males from other colonies. Mated queens are prevented from going into diapause by giving them a CO2 narcosis twice (2x 1/2 hour) (Röseler 1985). After narcosis the young queens can be put into cages and the whole process starts again.
The colonies used for pollination are provided with isolation material and are transported to the grower. Transportation for a longer period caused no problems. Enough food is stored inside the colony for at least 12 hours. Colonies were placed in the glasshouse, next to the main path, so that feeding and handling can be done easily. Direct sunshine on the colonies was prevented to protect the colonies from overheating. The hives were placed on top of a stand which was provided with an anttrap. The foot of the stand was therefore surrounded with water, to prevent ants from entering the hive and disturbing the colony. Hives must be provided with sugarwater immediately after placing. After a short while the entrance is opened so that the bees are able to orientate themselves and start visiting flowers. Bumblebee hives in a tomatoglasshouse must be provided continuously with sugarwater because tomatoflowers only have pollen available and no nectar. On average a number of 10 hives per hectare is sufficient for pollination. Certain types of tomatoes, such as cherrytomatoes have more flowers to pollinate and therefore more colonies are needed for pollination.
Colonies remain active for about 8 weeks. So to ensure pollination for the whole flowering period, colonies were added and/or replaced regularly. It can easily be seen if bumblebees have visited a tomatoflower. Flowers will get a brownish colouring soon after a bumble bee has visited it. When the bee grabs the flower, the anthers will be damaged a little on the outside. Growers can inspect if sufficient pollination has occurred. Once the flowers have withered, 100% should be visited. When this is not the case, an insufficient number of bumble bees could be the cause, or conditions for the plant are unfavourable so that the flowers become unattractive for the bumblebees to visit.

RESULTS AND DISCUSSION

At this moment we have reared bumblebee colonies continuously for 3 years. It is possible to build up and maintain a colony production starting with only a limited number of queens caught in the field. A management system for bumblebee colonies has been developed to realise pollination of tomatoflowers during the whole flowering season.

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POLLINATION OF THE RARE SPECIES *PHYTEUMA NIGRUM* (CAMPANULACEAE): FLIGHT DISTANCES OF BUMBLEBEES

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Keywords:
Phyteuma nigrum, bumblebees, phenology, mark-recapture.

Summary

Pollination ecology is included in the project studying the minimal requirements for the survival of populations of rare plant species. Bumblebee visitation of Phyteuma nigrum was studied in several populations in the Netherlands in 1989 and 1990. Flowering time and bumblebee species differed between the two years. In 1990 Bombus pascuorum queens were rather important visitors along with workers of B. pratorum and B. jonellus; the last two species were the most important bumblebee visitors in 1989. Mean flight distances between two inflorescences was 1.0 m, but 11 % of the flown distances were larger than 6.3 m. Mark- recapture indicated that queens of B. pascuorum were less site specific than workers of the other two bumblebee species.The percentage of recaptures in other patches was higher (70 % for B. pascuorum and 50 % for B. pratorum and B. jonellus. The maximum observed distance between two recaptures was 250 m. Although one isolated patch was within this range, at a distance of 150 m, those P. nigrum plants were visited by a completely different bumblebee population. The presence of other flowering plant species, important as food source for bumblebees, is discussed in relation to visitation and isolation of (small) P. nigrum populations.

INTRODUCTION

Today numbers and sizes of populations of many species decline due to changes in natural conditions or as a result of human activity; large populations become fragmentated or sometimes populations locally become extinct. In small (declining) populations of outbreeding species seed set may be reduced due to lack of pollinators and/or to a low level of genetic variation. Rare species often occur in isolated populations in nature reserves; they may show a decrease in genetic variation (Ouborg 1988). Therefore, pollination ecology is included in our project studying the minimal requirements for the survival of populations of locally rare plant species. The species studied are Phyteuma nigrum F.W. Schmidt, dark violet rampion (Campanulaceae) and Primula vulgaris Huds., primrose (Primulaceae). These species are mentioned on the Dutch red list (I.U.C.N.) as vulnerable (Phyteuma) and rare (Primula) (Weeda et al 1990). Both species are perennial and outbreeding, thus pollinators are important both for seed set and pollen exchange.

Pollen dispersal in insect pollinated species usually occurs over short distances, although some pollen is transported beyond the bounds of the populations (Levin 1986). The restricted spatial pattern is a consequence of pollinator foraging behaviour. Bumblebees and bees (butterflies to a somewhat lesser extent) tend to move from a plant to one of its neighbours, resulting in a leptokurtic pollen flow transport pattern.

Anything which alters the behaviour of the pollinators, will alter pollen dispersal in turn. When resources (pollen and nectar) are abundant, insects tend to be "held" in populations, and carry out large numbers of pollinations. When populations are small or have few flowers (early/late in flowering period) then pollinators are less site constant (Levin 1986). As floral resources become very abundant, then eventually the available pollinator pool may be saturated and visitation per flower will decrease (Rathke 1983).

In this paper we present data of flight distances between succesively visited inflorescences and flight distances between recaptures of marked bumblebees visiting P. nigrum. Bumblebees are considered most important pollinators. The results are discussed in relation to the possibility of pollen exchange between populations.

MATERIAL AND METHODS

The genus Phyteuma (24 species, Damboldt 1976) is entirely European in its geographical distribution. The vast majority of species is concentrated in the Alps. The floral biology of Phyteuma was already studied by Sprengel in 1873. Most recently Kovanda (1981) supported his observations: bees, bumblebees, butterflies and flies are visitors. The species seems to be visited primarily for nectar.

P. nigrum is a perennnial; the swollen roots survive the winter. Flowering is end May and June. The inflorescences (1-20) are spicate, ovoid to cylindrical and considerably elongated in fruit; they bear on average 40 flowers. The corolla is dark violet, sometimes bluish or white. Pollen is mostly dark red coloured. Flowers are protandrous, first presenting pollen and nectar. Nectar is produced by an epigynous disc at the base of the corolla. After the strictly male phase, the style elongates and the stigma begins to spread out (female phase) meanwhile the separation of the corolla slips continues up to the top of the tube. In a single inflorescence flowers in male and female phase are present, the male phase flowers arranged above the female phase flowers.

Observations were made in meadows in the state nature reserve "Stroomdallandschap Drentsche Aa", 30 km South of Groningen (the Netherlands). Phenology was measured as number of flowering inflorescences within three plots (4 m^2) in 1989 and in two plots (20 m^2) in 1990 in the same meadow. Flight distances between two inflorescences were measured in 1990, following workers of B. pratorum visiting P. nigrum at site Populierenlaan. In this project we are especially interested in the possibility of pollen exchange between P. nigrum plants occurring in different meadows. Therefore several neighbouring meadows were divided in plots of 10 x 10 m in May 1990, to facilitate position finding. In the field the position of bumblebees was noted with respect to the closest corner of the 10 x 10 plots. These meadows (Populierenlaan) are all situated along a river and about 50 m wide; the longest distance between the northern and southern plots was 330 m. Also bumblebees in a patch 140 m NE of Populierenlaan were observed (Meander). Bumblebees were individually marked with quick drying paint and without anaesthetic. Foraging characteristics and position in the field were noted when the bumblebees were observed. In 1989 bumblebee marking was done on a small scale (15 individuals). In 1990 more bumblebees were marked: 129 individuals. On three days (9, 16 and 23 May 1990) bumblebees were observed the whole day from 9.00 till 16.30 hours by four persons simultaneously in the different meadows. If bumblebees, marked in one patch were observed in another or vice versa then the possibility of pollen exchange between plants in these patches exists.

RESULTS

Flower phenology and visitors

P.nigrum started to flower early both in 1989 and 1990, due to a warm winter and early spring. Peak flowering in 1990 was even ten days earlier than in 1989 (Fig. 1). The flowering season lasted only 21-25 days. Few other bumblebee plant species were present: Ajuga reptans started to flower earlier and the species Trifolium pratense, Symphytum officinale and Rhinanthus angustifolius had their peak flowering later than P. nigrum.

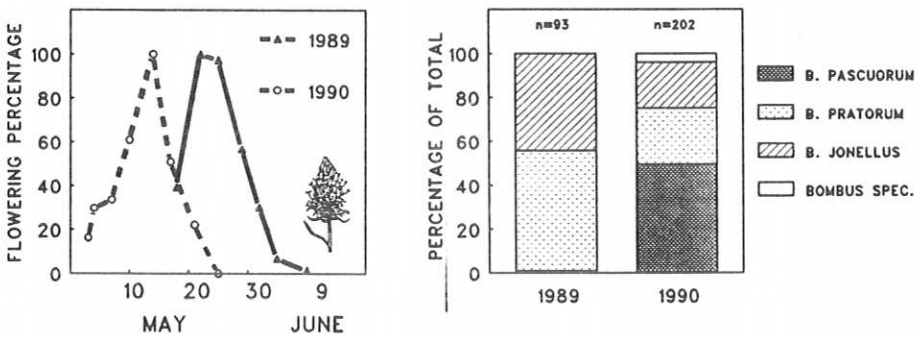

Figure 1: Flower phenology of a P. nigrum population in two years. Day with highest number of flowering inflorescences is 100 %.

Figure 2: Frequency distributions of bumblebee species visiting Phyteuma nigrum in two years.

P. nigrum in the Netherlands was visited by several bumblebee species (Bombus pratorum L., B. jonellus (Kirby), B. pascuorum (Scopoli)(Bombus taxonomy follows van der Blom (1989). Cuckoo bees (Psithyrus spec); Apis mellifera mellifera L., a small bee species and butterflies were occasional visitors. Syrphids (Rhingia campestris Mg.) were numerous, foraging for pollen and nectar. Their pollination efficiency will be discussed in another paper.

The workers of the short-tongued species B. pratorum and B. jonellus collected pollen and nectar in different ways. Nectar was collected in the same way as the queens of B. pascuorum did: the tongue was directly introduced between the slips of the corolla at the base of the flower. Pollen was collected on male flowers while hanging on the style. The slips of the corolla were pushed downwards by the legs and a new part of the style with fresh pollen became available for collection. Pollen was also deposited on the ventral side of the body and finally collected in the corbiculae.

The main difference between 1989 and 1990 in bumblebee species is the visitation by B. pascuorum queens in 1990 (Fig.2).

Some individuals were observed early to visit Ajuga, switched to P. nigrum and finally to Rhinanthus. The queens of B. pascuorum had just started their nest.

Flight distances between inflorescences

The flight distances of ten B. pratorum workers between two visited P. nigrum inflorescences were measured. Most distances were short; 62 % was shorter than 0.3 m (Fig 3). The mean flight distance was 1.0 m (N=91). The tail of the graph is long: 11 % of the flown distances is longer than 6.3 m and some of the observed distances were

longer than 20 m. Bumblebees flying these long distances were outside the range of vision of the observer.

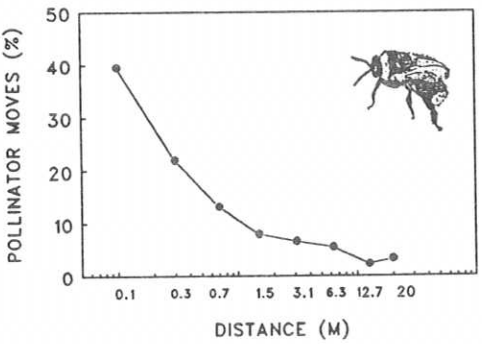

Figure 3: Pollinator foraging distances of B. pratorum workers visiting Phyteuma nigrum, as the frequency of total moves to a given distance (n = 91).

Recaptures within and between meadows

In 1989 a small number (n=15) of bumblebees visiting P. nigrum were marked (mainly B. pratorum and B. jonellus workers) in the meadows Populierenlaan. 60 % of the individuals were recaptured; 70.6 % of the recaptures were within the same meadow. In 1990 96 individuals were marked in the same meadows and 53.6 % were recaptured. B. pascuorum queens were responsible for 53 % of all recaptures in site Populierenlaan; the remaining 47 % were presented equally by B. pratorum and B. jonellus workers. Different species showed different site fidelities. On a small scale B. pratorum and B. jonellus were recaptured for about 50 % in the same or neighbouring plot (plot size 5 x 5 m), but B. pascuorum only for 30 %. On a larger scale B. pratorum and B. jonellus were also more constant to a particular meadow than B. pascuorum.

The largest distance between two recaptures on the same day was 80 m. Recaptures on the same day between meadows, making pollen exchange between patches possible, were observed early and late in the season to a greater extent than at the peak of flowering.

Distances between recaptures on different days were larger with a maximum of 250 m. Individuals marked at the site Populierenlaan were not recaptured at the site Meander and vice versa although the two sites were within this flight distance (150 m). Therefore we concluded that P. nigrum plants growing at these two sites were isolated from each other.

DISCUSSION

P. nigrum is an early flowering perennial, attractive both for its pollen and nectar. Bumblebees, workers of B. pratorum and B. jonellus and queens of B. pascuorum, are regular visitors.

1989 and 1990 differed in their flower phenology. Early flowering in 1990 resulted in the visitation of queens. In other years (1989 and 1987) queens only occasionally visited P. nigrum.

Bumblebee species differed in site specificities and in flight distances between recaptures. Queens were less constant to a particular meadow than workers. Bowers (1985) also found that workers appear not to utilize flowers outside the meadow where

their colony is located. Brian (1954) mentioned that bumblebees foraged only 19 m away from the nest. Heinrich (1975) stated that the costs in terms of time and energy promote this site specificity. Combined with the foraging behaviour of majoring and minoring of bumblebees (Heinrich 1979) site specificity may play an important role. P. nigrum populations are not very large, with some dense patches and a maximum of thousand inflorescences per meadow. Another attractive bumblebee plant Rhinanthus angustifolius was present with increasing numbers of flowers during the anthesis of P. nigrum. Bumblebees regularly visited P. nigrum and Rhinanthus on one foraging trip and pollen of Rhinanthus were often found in the corbiculae. The presence of Rhinanthus may increase site specifity and may promote visitation of P. nigrum patches within meadows, but Rhinanthus may also be visited between P. nigrum patches in different meadows. On the other hand the possibility of improper pollen transfer exists if P. nigrum and Rhinanthus are visited on the same foraging trip.

P. nigrum plants at site Meander were isolated from plants in site Populierenlaan by an area empty of flowering plants. Although within flight distance of bumblebees both sites had their own bumblebee fauna. The absence of food plants perhaps prohibited bumblebees to cross this empty area. Thus both sites can be considered as two isolated populations, assuming that gene flow by seed dispersal is unlikely to occur.

Our results showed that years may differ in bumblebee species and in the resulting pollen flow. Early flowering of P. nigrum promotes pollenflow between patches, due to visitation of queens instead of workers, assuming that bumblebees still carry fertile P. nigrum pollen. In general pollenflow is restricted. In Glechoma hederacea fruitset in female clones, isolated from the nearest hermafrodites by more than 200 m was 0 %, isolated c. 100 m, fruitset was very low (1 %)(Widen and Widen 1990). Due to the fact that not all pollen grains from the first flower are deposited on the second one but that at least several flowers will receive pollen (pollen carry-over, Lertzman and Gass 1983) one single recapture between meadows may result in pollination of several flowers with pollen from the other meadow. A low level of crossing between patches (populations) may be sufficient to prevent the negative effects of inbreeding.

The presence of other flowering species, attractive for bumblebees may influence the visitation of small patches (populations), by increasing the site attractivity. On the other hand if many flowers of other species are present, all pollinations of P. nigrum will be within a restricted area thus increasing the risk of genetic erosion. The variability in phenology and the resulting visitation by various species of bumblebees may counteract this problem.

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THE MANAGEMENT OF *OSMIA RUFA* L. FOR POLLINATION OF SEED CROPS IN GREENHOUSES

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Keywords:
Osmia rufa, management, hibernation.

Summary

The management of the solitary bee Osmia rufa L. for pollination of seed crops in greenhouses has been studied. The study was focused on the relation between the length of the hibernation period and the time needed for activation after the hibernation.

It was shown that Osmia rufa, under the described circumstances, has a minimal and a maximal hibernation period. The optimal duration of the hibernation is roughly between 120 and 170 days. A substantial part of the bees die during activation when the hibernation period was too short or too long, the emerged bees live short and are not active in the crop. After an optimal hibernation period the bees are active in the crop in case the emerged bees are stored at 5 °C. for a maximum of 4 days.

There is a correlation between the length of the hibernation period and the emergence of bees. Bees emerge within 14 days after the emergence of the first bees. In this period no parasites emerge. These phenomena make it possible to attune the activation of the bees to the flowering period of the crop.

INTRODUCTION

In 1989, in co-operation with the Dutch Horticulture Seed Firms (NTZ) a research project was started to develop a managementsystem of the solitary bee Osmia rufa L. for seed crop pollination in greenhouses. There were two reasons for this project:

1. The availability of honey bees is, due to bee diseases, not always assured.
2. Particulary in small compartments, unlike honeybees, solitary bees can be used in small amounts. The number of pollinating insects can be attuned to the number of flowers to be pollinated.

The solitary bee Osmia rufa is used for this project because this insect is common in the Netherlands and because they are easy to collect. In several institutes abroad work has been done on Osmia species and good results have been obtained.

In nature Osmia rufa L. starts its activities in March - April. The male bee emerges some days previous to the female bee. The bees mate and the female bee starts to nest in tunnels. The development of

the brood takes about 100 days. The adult bee hibernates inside the cocoon. This makes it possible to collect the cocoon in the autum, hibernate the bees in the refrigerator and activate the bees in spring.

The research was focused on:

a. The relation between the length of the hibernation period and the time needed for activation after hibernation.
b. The practical management of Osmia rufa in greenhouses for pollination purposes.

MATERIAL AND METHODS

a. The relation between the length of the hibernation period and the time needed for activation after the hibernation.

On the 9th of November 1989 cocoons were taken out of the nests, divided in two groups (cocoons of 8 mm long which contained probably males and cocoons of 10 mm long with females) and placed in the refrigerator at 5 °C. After the hibernation on 9 different dates cocoons were transferred to gelatine capsules (size 00) and placed in an incubator at 20 °C. to be activated. Each group holded 50% 8 mm cocoons and 50% 10 mm cocoons. The time it took the male and female bees to emerge was recorded. At least 14 days after the emergence of the first bee, the still closed cocoons were opened and checked.

b. The practical management of Osmia rufa in greenhouses for pollination purposes.

The dates the bees were activated were linked to the expected flowering period of the crop. Due to wheather condition the flowering of the crop was often delayed. This period was tide over by placing the bees, immediately after the emergence and before having dropped the meconium, in a refrigerator at 5 °C. As soon the crop flowered the bees were placed in the compartments in greenhouses. In the compartments artificial nests were placed to let the bees nest.

RESULTS AND DISCUSSION

a. The relation between the length of the hibernation period and the time needed for activation after hibernation.

- Emergence and failure rate after different hibernation periods.

The results are given in table 1

table 1: Emergence and failure rate after different hibernation periods

hibernation period (days)	n cocoons (8+10 mm)	percentage emerged cocoons	percentage not emerged cocoons	percentage cocoons with parasites
74	360	33%	66%	1%
84	87	16%	77%	7%
96	85	19%	81%	0%
109	193	40%	49%	11%
126	839	93%	1%	6%
166	279	97%	1%	2%
174	308	86%	12%	2%
192	135	30%	69%	1%
210	251	48%	48%	4%

The data show clearly that, under the described circumstances, the hibernation period can be too short and too long. The minimal period appears to be somewhere between 109 and 126 days. The maximal period ends somewhere between 166 and 174 days. The major part of the bees which remained inside the cocoons after the activation period, was dead. These bees had died during the activation.

- Activation period of male and female bees.
The data are given in table 2 and 3.
table 2: Mean activation period of male bees after different hibernation periods.

hibernation (days)	n male bees	mean activation-period (days)	sd	days till first emergence	days till last emergence
74	65	25	5	18	34
84	9	14	1	11	15
96	15	10	3	5	13
109	43	12	3	7	20
126	**414**	**9**	**2**	**6**	**18**
166	**155**	**4**	**1**	**2**	**9**
174	147	4	1	2	12
192	16	4	1	3	6
210	94	1	1	1	5

table 3: Mean activation period of female bees after different hibernation periods.

hibernation (days)	n female bees	mean activation-period (days)	sd	days till first emergence	days till last emergence
74	53	28	3	22	33
84	5	19	1	18	21
109	34	16	3	11	22
126	**350**	**15**	**3**	**10**	**22**
166	**112**	**7**	**1**	**5**	**10**
174	118	6	1	4	7
192	24	7	2	5	11
210	26	3	1	2	4

The data show a correlation between the hibernation period and the time needed for activation. The period of 14 days, taken after the emergence of the first bee appears to be long enough. In this 14 day period all vital bees have emerged and no parasites did emerge. In the cocoons, opened after this 14 days period, the parasites Monodontomerus obscurus and Anthrax anthrax were found.

b. The practical management of Osmia rufa in greenhouses for pollination purposes.
- Results after the short hibernation periods of 74, 84, 96 and 109 days.
The activity of each group corresponded with each other. The emerged bees were stored at 5 °C. for at least 5 days and subsequently placed in the compartments. These bees were only some days active in the compartment. No bees were observed, visiting the flowers to collect pollen and no bee started to nest.

- The results after the hibernation period of 126 and 166 days.
The vitality and activity of the groups corresponded with each other. After both hibernation periods only 1% of the bees died during activation. The emerged bees stored at 5 °C. for a maximum of 4 days or placed in the crop immediately after emergence were active, collected pollen and nested. The major part of the bees, stored after activation for periods of 7 days or more died in the compartment within some days. It is known that Osmia lignaria can be stored after activation for maximal 7 days. Longer period affects the vitality. (personal communication Torchio). The same phenomenon is seen in our observations. It took the females, hibernated for 126 days about 7 days before they started to collect pollen. For the females, hibernated 166 days this retention period was 3 days.

- The results after the long hibernation of 174 and 210 days.
After the 174 day hibernation female bees were stored after activation for 24 days. The bees died within some days in the crop. After a storage of 10 days part of the bees died within some days. The rest was active in the crop. After the 210 day hibernation the females were stored for 5 days. After having placed these bees in the greenhouses, part of the bees died. The surviving part was active in the crop, collected pollen and started to nest.

CONCLUSION

a. The relation between the length of the hibernation period and the time needed for activation after hibernation.

- The hibernation period is limited. In case the bees are hibernated too short or too long a substantial part of the bees die during activation.
- The optimal hibernation period is roughly between 120 and 170 days.
- The majority of the bees emerge within 14 days after the emergence of the first bees. In this 14 day period no parasites emerge.
- The activation period of both males and females depend on the length of the hibernation period. The males have a shorter activation period than the females.
- The 8 mm cocoons contained 86 % males and the 10 mm cocoon 78 % females (emerged bees)

b. The practical management of Osmia rufa in greenhouses for pollination purposes.

- The bees are, after a too short or a too long hibernation period, not vital. Storage of these bees after activation at 5 °C. also has a negative effect on the vitality and foraging activities. These bees are not suitable for pollination work.
- The bees, emerged after the optimal hibernation period and stored at 5 °C. for at the most 4 days are active in the crop. They collect pollen and start to nest. A longer storage after activation shortens the lives and decreases the vitality of the bees.
- It takes, depending on the hibernation period some days before the bees start to collect pollen.
- Bees are active at temperatures of 15 °C. or more.

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LAYING WORKERS IN AFRICANISED HONEYBEES

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Keywords:
Honeybees, colony reproduction, africanised bees, population biology, sociobiology.

Summary
Egg laying by workers is much more common in africanised honeybees than in European honeybees. This is illustrated with a number of observations. We discuss the adaptive significance of this phenomenon for both *Apis* races. Workers who develop into laying workers seem to be involved with the raising of emergency queen cells slightly more than others.

INTRODUCTION

Just as in any other tropical country in Latin America, africanised honeybees dominated the local population of *Apis mellifera* in Costa Rica within a few years after their arrival in 1983. The ecology of these africanised honeybees and their competitive advantages over the formerly present honeybees of European origin have been reviewed by Roubik (1989). The africanised honeybee multiplies rapidly through excessive swarming (Otis, 1982) and completely dominates the production of males in a mixed population (Rinderer et al., 1985).

Africanised bees in South and Central America originated from *A.m. scutellata* queens that were imported into Brazil in 1956. In Costa Rica, the africanised bees can not be considered to be purely *A. m. scutellata*, since they have mixed with other types of honeybees that were present. According to Ruttner & Hesse (1981) egg laying workers can be found in colonies of this African race 7 to 13 days after becoming queenless (average 9.5 days). This is a much shorter period than was found for colonies of four European races (from 16 to 30 days). In this paper, we will discuss the idea that egg laying workers in africanised bees may contribute considerably to the production of males. This may be a very important factor involved in reproductive dominance within mixed populations of africanised and European honeybees.

The presence of laying workers in a colony can easily be recognised by the irregular pattern of the eggs in the cells. Many eggs can be found in one single cell, and many of these eggs are not laid at the bottom of the cell, but on one of the walls (Fig. 1).

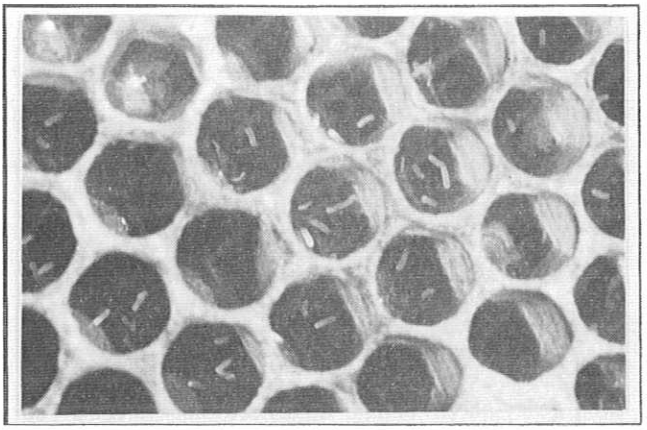

Figure 1
Worker laid eggs. The colony from which this comb was taken also contained 8 closed queen cells (Table 1, Hive 4).

METHODS

Ruttner & Hesse (1981) tested queenless hives with a small amount of old brood, so no emergency queen rearing could take place. In our work with africanised bees we did not only observe the development of egg laying workers under these 'hopelessly queenless' situations, but also in colonies that were rearing replacement queens.

Two africanised colonies were housed in observation hives so that the behaviour of individually marked, similarly aged workers could be followed. During daily periods of two to four hours of continuous observation, the number of body insertions of each marked worker into emergency queen cells was recorded (the insertions scored lasted longer than five seconds, deep enough to reach the larva at the bottom of the cell). Shortly after the first laying workers were observed, all marked workers were dissected to determine their level of ovarian activation. Individual involvement in attending queen cells was correlated to ovarian activation by means of Spearman rank correlation tests.

RESULTS AND DISCUSSION

Examples of cases in which we found laying workers in africanised beecolonies in relatively early stages are illustrated in Table 1. In several cases (colonies 2, 3 and 4), laying workers were present in considerable numbers at the same time as queen cells containing larvae or pupae. Furthermore, we were often able to determine that laying workers were present very quickly after their colony became queenless. The data from this table show that the development of laying workers in africanised bees is so rapid that they can take advantage of very short queen-less periods to produce some males, even if these periods are no longer than necessary for a natural change of queens. This does not happen in European honeybees. The time needed for complete ovarian activation in the European races is so long that laying workers will only be present in situations where there is no further chance that a replacement queen will emerge in the same colony (Ruttner & Hesse, 1981). In mixed populations this means that africanised workers in temporarily queenless colonies contribute to the production of males, whereas European workers do not in the same circumstances.

Table 1

Colony	Date	(Presumed) no. of days queenless	Observed phenomenon
1	1983	3 - ?	First swarm of africanised bees found by Arce in the town of Heredia. Three days after the arrival of the swarm the bees had constructed a small comb in which many worker laid eggs were found. The swarm was queenless.
2	Febr. '90	5	Small observation hive (±1500 workers) from which the queen was removed. The workers started emergency queen rearing, and closed 4 queen cells. The first laying worker was observed 5 days after the queen was removed, before the last queen cell was closed.
3	March '90	5	Small observation hive (±1500 workers). Two days before the queen was removed, the colony attempted to abscond, but the queen was caught and put in a small cage in the hive. The first laying worker was observed 5 days after subsequent queen removal. Six queen cells were closed, 5 after the first laying worker was seen.
4	March '90	8 - 12	Colony which lost its queen. Laying workers were present in great numbers (see Fig. 1) while at the same time 8 closed queen cells with white pupae were found. No virgin queens had emerged yet.
5	Several times	0 - 3	Swarm in which egg-laying workers were found, together with a virgin queen.

There are no indications that the development of laying workers interferes negatively with the raising of new queens. The experience of bee-keepers is that africanised colonies are excellent nursing colonies for queen rearing. Apart from that, the number of emergency queen cells made after a queen dies is not known to be lower in africanised than in European bees (As far as we know, there are no systematic data on this). The correlations between ovarian activation and the number of body insertions into queen cells by each individual worker are shown in Table 2. In this table, we give results of the analysis of Hives 2 and 3 (from Table 1) and also for four colonies of European honeybees. In all colonies we found positive correlations between the frequency of visits to larvae in queen cells and ovarian activation, although this is significant in only one colony (africanised bees, Hive 3). Future laying workers thus seem to be involved in queen rearing slightly more than workers without ovarian activation, although on the whole this tendency is weak.

The development of laying workers is attended by a great deal of violence inside the

hive. In general, the victims are the workers with activated ovaries, whereas the aggressors havenot undergone ovarian activation. During aggressive encounters, workers are sometimes killed or mutilated by their nestmates, although on the whole this occurs rarely. A much more common physical result of aggression is that the victim donates food in large quantities to other passing workers. These trophallactic interactions seem to be important in the competition for a limited supply of proteins. Only a small percentage of workers is able to gather enough protein to be able to finish the development of their eggs. Aggression thus works as a barrier for the accumulation of proteins in an individual. (This process is reported and discussed in detail by Van der Blom, in prep.). The hypothesis that egg laying by workers only occurs if there is an accumulation of scarce proteins is supported by the fact that hardly any laying workers develop in presence of a great number of larvae (Hess, 1942 and Müssbichler, 1952), even if thereare rich stores of pollen and honey in the nest. In all cases mentioned in Table 1, there had been a period with few or no larvae prersent.

As illustrated here, africanised bees manage to allocate proteins for the development of eggs much more quickly than European bees. The explanation of this probably has to be sought in the ecology of the different races in their original habitats. As mentioned in the introduction, africanised bees swarm frequently. In Costa Rica, as is probably the case in most tropical countries, swarming occurs in all times of the year, with some decrease during the raininy period from (September to November). Drone brood can also be found all year, with a peak during the dry season. Since reproduction can take place at any time, it may be worthwhile for the workers to produce drones as soon as there is a chance, i.e. if there is a period without the pheromonal influences of a laying queen and with a small quantity of larvae to be fed. The worker produced drones may encounter a queen for mating at any time

Table 2

Hive A = Afr. E = Eur.	Final no. of marked workers	No. of body insertions into queen cells	Results of the Spearman rank correlation test	
			Z(corr.)	P
2 A	161	132	+ 1.306	> .1
3 A	170	135	+ 3.162	.0016**
1 E	180	162	+ 1.362	> .1
2 E	133	321	+ 1.389	> .1
3 E	121	216	+ 1.658	.097
4 E	151	112	+ 0.422	> .1

Correlations between ovarian activation and visiting of queen cells

Visits to queen cells were observed during daily periods of several hours of continuous observation. Only the workers that penetrated into the cell far enough to reach the larvae for at least five seconds were counted. Unfortunately we do not know exactly what the bees did inside the cell. Not all bees will have been feeding the queen larvae. (Perhaps some of them even ate from the royal jelly themselves!)

of the year.

European honeybees, on the other hand, have been adapted to a completely different annual cycle. Successful reproduction in Europe only occurs during a short period in spring. The production of drones is limited to a few months of the year; any male that is produced outside this period has no chance to reproduce successfully and can thus be considered as a waste of energy. If a colony becomes queenless after swarming, it takes about six weeks before the first worker produced males will be ready to fertilize queens (a minimum of 10 days for workers to produce eggs + 21 days of developmental time for the drone + 11 days for the adult drone to mature = 42 days). These males thus appear at least about one and a half month after the peak in the swarming season, and will only be able to encounter queens from the few colonies that swarm (or lose their queen) late in summer. Many of these colonies will have problems later on, since they may not be able to raise enough bees to survive the winter season.

Even if laying workers are present in Europe, it is not very likely that their sons mate with a queen that subsequently produces a viable colony. The workers in africanised colonies profit from any queenless circumstances by producing males, which all have chances to mate successfully.

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RHYTMIC PATTERNS OF STROKING BEHAVIOUR OF WORKERS IN *TETRAGONISCA ANGUSTULA* (APIDAE: MELIPONINAE)

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Keywords:
Tetragonisca angustula, rhytmic patterns, stroking behaviour, wax scales.

Summary
In *Tetragonisca angustula* a rhythmical pattern of stroking by workers occurs on the upper combs which is synchronized with the ovipositionperiods of the queen. In the same area where this pattern of typical worker behaviour occurs, wax scales appear on the surface of the upper combs in a similar progress.

Introduction

As other stingless bees, *Tetragonisca angustula* shows a rhythmic egglaying pattern: periods of queen ovipositions alternate with periods of building broodcells [1,2].
Only during the oviposition periods the queen is present on the comb. In a short period a batch of broodcells is filled with larval food by workers, after which the queen lays her egg on the larval food. After her last oviposition the queen leaves the combs which then demarcates the transformation from a short egglaying period to a longer period of exclusive cellbuilding.
In *Tetragonisca angustula* the construction pattern of broodcells is successive: at every moment cells of different stadia are present.

Preliminary observations revealed two remarkable fenomena:

1. workers on the comb show typical stroking behaviour
2. wax scales are found on the surface of the comb in areas with stroking behaviour.

To analyze the function of stroking by workers, we studied the occurence of this behaviour and its relation to the rhythmic egglaying pattern and the appearance of wax structures on the comb.

Materials and methods

Observations of behaviour were made by means of video. The following recordings were made:
-The occurrence of stroking behaviour as well as the presence of wax particles on the upper two combs was registered in separate one minute-blocks prior to or subsequent to the last oviposition of the queen in a cellbatch.

Results

A The typical stroking behaviour of workers on the comb consists of a pronounced stroking with one or two hindlegs over the dorsal, lateral and ventral side of the abdomen. In addition, they stroke their hindlegs while raising the abdomen.

B The stroking behaviour of workers on the comb occurs in a rhythmic pattern. The average number of workers that display stroking behaviour on the combs is indicated in

chart 1. During the end and shortly after the process of provisioning and oviposition this behaviour is at its maximum. It is clear that during the period of exclusive cellbuilding the stroking behaviour on the comb deminishes.

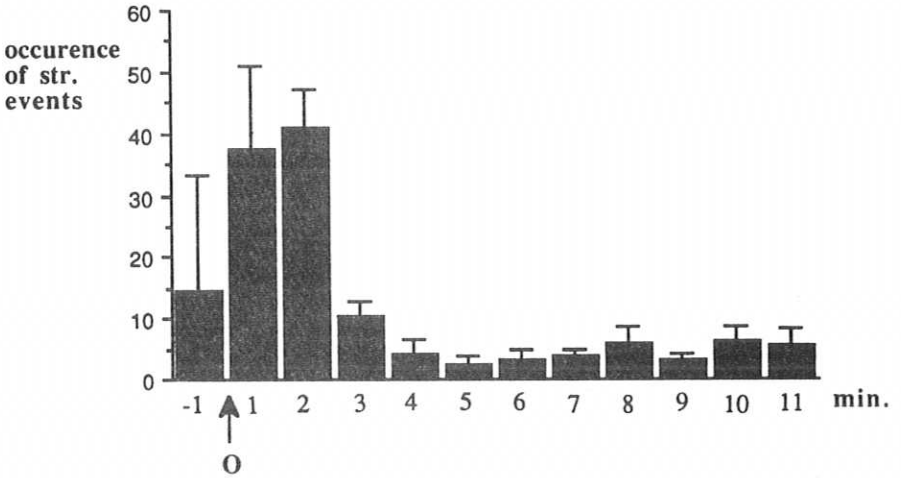

Figure 1: a pre-post state histogram of stroking behaviour on the comb at successive one minute lags prior to or subsequent to **O**: the last oviposition of the queen in a cellbatch. Indicated are the average values for three successive oviposition/cellbuilding periods.

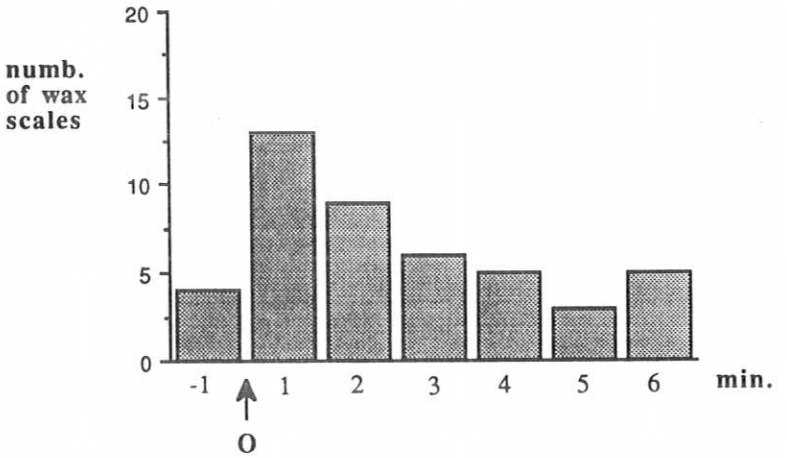

Figure 2: a pre-post state histogram showing the summed number of wax scales present on the comb at successive one minute lags prior to or subsequent to **O**: the last oviposition of the queen in a cellbatch. The observation period consisted of six hours (n=3).

C Correlated with the rhythmic occurence of stroking behaviour there is a rhythmic pattern in the temporal appearance of wax scales on the comb. Our data confirm the appearance of wax scales generally just after the last oviposition of the queen in a cellbatch (figure 2). During the period of exclusive cellbuilding the number of wax

scales on the comb declines. The deposition of some wax scales could be determined as the direct result of stroking behaviour.
Our data reveal the appearance of 27.3 wax scales per six hour period (SD=26.1, n=3). The scales lying on the comb have a oval to multisquare form and vary in size from 0.42 x 0.33 mm to 0.50 x 0.62 mm (figure 3).

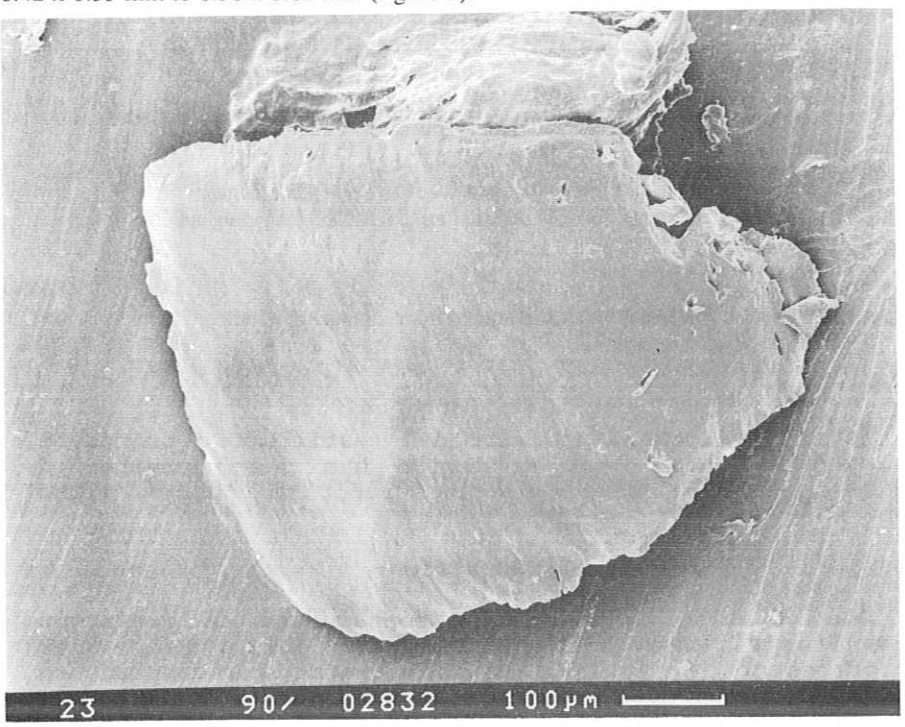

Figure 3: a SEM photograph of a wax scale collected from one of the upper combs in a *Tetragonisca angustula* colony

Conclusions

*The stroking by workers shows a rhythmic pattern, synchronized with the ovipositionperiods of the queen.

*This typical worker behaviour is at its maximum just when a oviposition period alternates with a period of exclusive cellbuilding.

*The appearance of the wax scales on the comb shows a similar progress during this period.

*The relative small number of wax scales appearing on the comb, compared to the intensity of stroking indicates that stroking on the comb, in order to deposit buildingmaterial, is not very efficient.

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MONITORING HONEY-BEES: THE DESIGN OF A COMPUTER-OPERATED BEE COUNTER

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Keywords:
Apis mellifera, foraging behaviour, activity recorder, counting device.

Summary

After development and extended testing of a prototype an advanced bee counting device has been constructed which certainly deserves the predicate 'stand alone continuous monitoring field device'.

INTRODUCTION

Bee counting devices were constructed in different ways (for a review see RICKLI et al., 1989). This paper describes how a new type of bee counter was constructed and which requirements were posed when constructing it.

Following requirements were posed when designing the new beecounter:

- The counting device should be able to monitor a bee colony of full value in full activity.
- Bees should not experience any hindrance in their normal activities.
- Ventilation of the hive should be guaranteed.
- The outlook of the hive should not be changed in such a way that it troubles the orientation of the honeybees.
- Simultaneous registration of different colonies should be possible and the number of concurrent working counting devices should be extendable without limit.
- The counting device should be solid enough for outdoor experiments and should work completely autonomous.
- Finally the bee counter should be a user-friendly and low-maintenance construction.

Based on these requirements a prototype has been constructed. It has been fully tested and the results of these tests have led to the creation of a second, improved type of bee counter.

MATERIAL AND METHODS

Of many ways of detecting a passing bee the photoelectrical one causes no physical hindrance and is capable of making a distinction between incoming and outgoing bees.
When applying the photoelectrical detection method bees are forced to pass a tunnel and by doing this they interrupt a light beam.

Two light emitting diodes (LED, LR 3360 Siemens, peak emission 660 nm.) placed one after the other (6 mm. in between) in a rectangular tunnel (8 x 8 x 20 mm., see figure 1), generate two vertical red light beams. Thirty-two of such tunnels are placed one next to the other, covering in this way a complete hive entrance (length 42.5 cm). The LEDs are placed in the bottom of each tunnel. They are protected against pollution by a clear plexiglas which covers the bottom of the 32 tunnels.

In top of each tunnel two phototransistors (SFH 309 Siemens) are placed, which detect if a light beam is interrupted.
By placing two light beams one after the other it is possible, by a logic reasoning, to deduce in what direction a bee is passing. This logic deduction is executed at each tunnel by an intelligent In/Out detector and the result is passed on, via a demultiplexer, to a central microprocessor (8-bit control-oriented microcomputer 8751H Intel). The scanfrequency of each tunnel is 2000 Hz.

The bee counter is connected to an IBM-compatible Personal Computer (PC). The communication between both is effected by an internal modem in the bee counter and an external one coupled to the serial port of the PC. This allows the use of simple, low- cost, twisted-pair lines, which length can cover more than 500 meters, between both modems.

The software (own design) on the PC asks for the number of counted bees at a freely chosen interval and stores those numbers on diskette. The number of recordings that can be stored depends on the capacity of the storage medium. On a 3.5" diskette of 720 kB, 36000 recordings are stored. Using an interval of 1 minute, this means a 25 days continuous recording. The software performs a first processing of the data, and is able to generate a graph as well.

The prototype is using 220 V mains voltage as power source.
The housing of the counter consists of two parts. The bottom part contains the power supply (transformator and

stabilisator) and the light emitting diodes, with on top of them the clear plexiglas.
The upper part contains the phototransistors and the major part of the electronics. The profile of the tunnels is also incorporated in the upper part of the bee counter. Both parts are connected to each other by means of a hinge on one side and a lock on the other side. In this way tunnel profile and plexiglas are easily accessible for maintenance. For the prototype a wooden housing is used.

After development and construction in the course of 1988, extended tests on the prototype in the open, in glasshouses and in a BFR (Bee Flight Room, cfr. JACOBS & KELLNER, 1977) were executed in 1989. In these tests the device was submitted to extreme physical conditions and large numbers of bees.

RESULTS AND DISCUSSION

The design of the prototype appears to meet all the requirements that were posed. The results of the tests with the prototype however showed that some amendments could be added. These improvements have been used to build a new type of bee counter, called BeeScan

The intensity of the light beams had to be adjusted in function of the transmittance of a honeybee body.

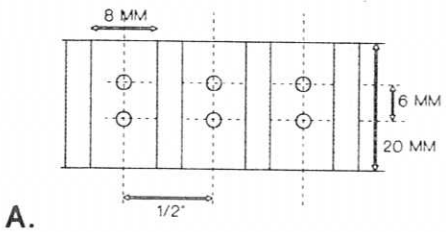
The shape of the tunnels had to be changed also. The new profile, which has a broader part on the exit side, and of which the ceiling is barrel-shaped (figure 2) forces the bees to pass exactly over the light beams and allows the guard bees to perform their normal behaviour without disturbing the recordings.

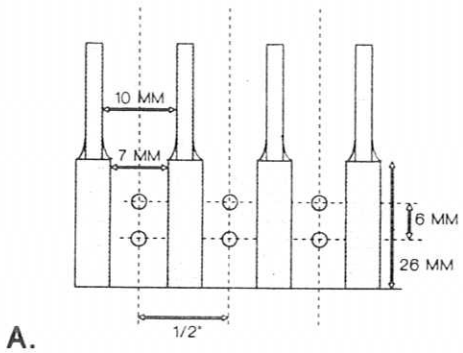
Heat production by the transformator in the lower part of the counter disturbed the normal behaviour of the bees. Therefore power supply was changed in such a way that whatever kind of 12 V power source can be used. This also improves the autonomy of the bee counter.

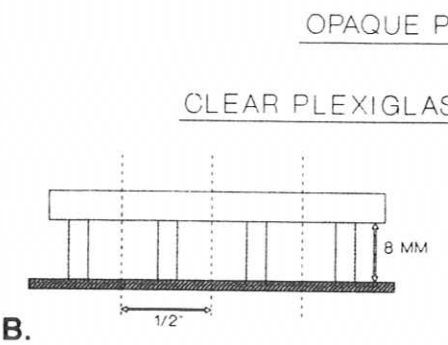
For the housing of the new bee counter a hardened, UV-resistant PVC is used. A more compact housing became possible by removing the transformator and applying the most recent developments in electronics (EPLD, electrical programmable logic device), the latter also reduces the power consumption of the counting device to less than 4 W.

Testing on a larger scale should prove if any amendments are still possible. Providing the bee counting device with a larger memory capacity and in this way reducing the communication with a PC to a minimum, could be one of these amendments. Simultaneous recording of abiotic

parameters such as temperature and humidity, by coupling sensors directly to the bee counter is another possibility.







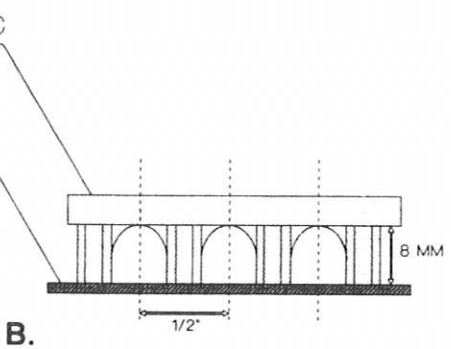


Figure 1. Topview (A) and frontview (B) of the tunnel profile of the bee counter prototype. The circles indicate the position of the LEDs.

Figure 2. Topview (A) and frontview (B) of the tunnel profile of the type 2 bee counter (BeeScan). At the exit side a broad extension is added. Top of the tunnels is changed to a barrel-shape.

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INVASION OF VARROA MITES INTO HONEYBEE BROOD CELLS; WHEN DO BROOD CELLS ATTRACT VARROA MITES?

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Keywords:
Apis mellifera, Varroa jacobsoni, parasite, brood invasion.

Summary

Invasion of Varroa mites into brood cells was studied in an observation hive, using combs with cells at one side only. The cell bottoms had been replaced by a transparent sheet, through which mites appeared to be clearly visible after invasion into a cell. Cells were invaded by the mites during a period preceding cell capping: 15-20 hours for worker brood and 40-50 hours for drone brood. The larger number of mites generally found in drone cells, when compared to worker cells, could be partly caused by the longer period of drone brood attractivity.

INTRODUCTION

The Varroa mite, *Varroa jacobsoni* Oud., is an important pest of the honeybee, *Apis mellifera* L. (Ritter, 1981; de Jong, 1984; Ifantidis & Rosenkranz, 1987). Female mites parasitize both adult bees and bee brood, but only reproduce in brood cells. The invasion of Varroa mites into brood cells is therefore a crucial phase determining reproductive success. Upon leaving the bee they may discriminate between worker and drone brood. It has often been suggested that mites prefer drone brood to worker brood, because larger numbers of mites are generally found in drone brood and the rate of reproduction is higher (Rosenkranz et al.,1984; Schulz, 1984).

Mites probably use signals to decide whether to invade a brood cell or not, possibly a signal coming from the larva. This signal could be different for worker and drone brood. Knowledge about the period during which brood is attractive, i.e. the period when signals can be expected to be present, is the first step towards their identification. Brood is attractive to mites just before cell capping (Ifantidis, 1988; Fuchs & Müller, 1988). However, when exactly is still unknown, making observations of mite invasion into brood cells necessary.

MATERIAL AND METHOD

A small colony of bees highly infested with mites was put into an observation hive, containing two combs with cells at one side only. The cell bottoms had been replaced by a transparent sheet. Mites that invaded cells immediately crawled behind

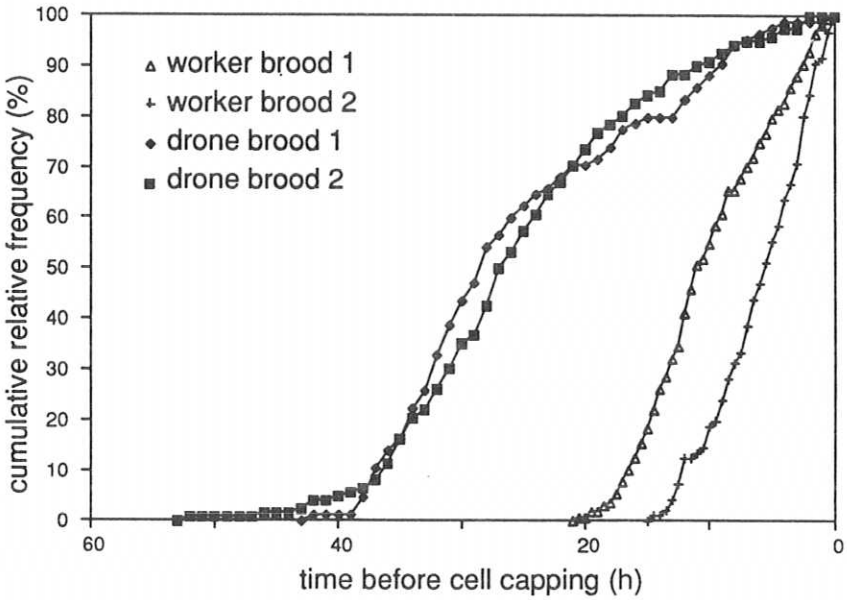

Figure 1: Cumulative relative frequency of mites invading honeybee brood cells preceding cell capping.

the larva and appeared to be clearly visible through the cell bottom. Brood cells were observed at half-hour intervals for worker cells and one-hour intervals for drone cells. For each cell, records were made of the time that mites had appeared and the time at which the cell had been capped. Two replicates with both worker and drone brood were made.

RESULTS AND DISCUSSION

When brood cells were invaded, the bee larva completely covered the cell bottom and the distance between larva and cell opening was decreasing as a result of larval growth. Cells were invaded by the mites during a limited period preceding cell capping: 15-20 hours for worker brood and 40-50 hours for drone brood (fig. 1). However, the numbers of invading mites were highest during the first half of this period. Probably most mites had already left the bees by the time brood cells were nearly capped.

Drone brood appeared to be attractive during a two times longer period than worker brood. This is in agreement with earlier studies providing indirect evidence. Ifantidis (1988) used the weight of larvae as an estimate of their age, and found that mites invaded into drone and worker brood cells from 45 hours and 15 hours before

cell capping, respectively. Fuchs & Müller (1988) found mites from about 60 hours and 30 hours before capping in drone and worker brood.

The larger number of mites generally found in drone cells, when compared to worker cells, could be partly caused by the longer attractive period. When trying to identify signals which play a role in brood cell invasion by mites, we need not necessarily expect different signals of worker and drone brood. In theory, it may be the same signal that causes attractivity to mites, both in worker and drone brood.

This research was supported by the Technology Foundation (STW), Utrecht, the Netherlands.

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MICROSPORIDIOSIS IN MASS-REARINGS OF THE PREDATORY MITES *AMBLYSEIUS CUCUMERIS* AND *A. BARKERI* (ACARINA: PHYTOSEIIDAE)

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Keywords:
Microspora, Pleistophoridae, *Amblyseius spp.*, pathology, transmission, mass-rearing.

SUMMARY

The commercial production of the predatory mites *Amblyseius cucumeris* and *A. barkeri,* used for biological control of thrips in vegetables in greenhouses, is at stake, because of a reduction in production and quality of these predatory mites in mass-rearings, due to the action of a pathogen (Microspora: Pleistophoridae). Aspects of the pathology and transmission are discussed.

INTRODUCTION

The predatory mites *Amblyseius cucumeris* (Oudemans) and *Amblyseius barkeri* (Hughes) are used for biological control of the thrips species *Frankliniella occidentalis* Pergande and *Thrips tabaci* Lindeman, both serious pests in greenhouse vegetables. The predators are part of the integrated pest management programmes of several vegetables in greenhouses in the Netherlands. For the commercial production of the predators, a mass rearing method is used, originally developed by Ramakers and van Lieburg (1982).

The predators are reared in large aerated containers, which are filled with hundreds of liters of wheat bran. A temperature of approximately 22 °C and a high relative humidity (~90%) allows the growth of fungi on the bran. The fungi serve as food for stored product mites (*Acarus siro* L. or *Tyrophagus putrescentiae* (Schrank) and *Tyrolichus casei* (Schrank)), which in turn are fed upon by the predatory mites.

Recently, the productivity of the mass rearing decreased drastically as a consequence of a disease in these *Amblyseius* species (Ramakers *et al.*, 1989; M. Dissevelt & W. Ravensberg (Koppert B.V., pers. comm.). Not only productivity, but also quality of the predators produced may have been reduced. For these reasons, the disease is a threat for the biological control of thrips. If pesticides (such as pyrethroids) have to be used instead, the full program of integrated pest control measures is at stake, since the pesticides may interfere with the effectivity of other natural enemies.

DIAGNOSIS

The pathogen causing the disease belongs to the Microspora. Ramakers *et al.* (1989) suspected that the pathogen is a member of the genus *Nosema*, but more detailed investigations showed that we are probably dealing with a new species belonging to the genus *Pleistophora* (A.M. Huger (Institut für Schädlingsbekämpfung, Darmstadt, B.R.D), pers. comm.).

Microsporidia belong to the phylum Microspora, which is one of the 7 recently erected phyla within the Protozoa (Levine *et al.*, 1980). They are common pathogens of invertebrates, but can be found in vertebrates as well (Canning, 1971; Hazard *et al.*, 1981).

BIOLOGY OF MICROSPORIDIA

Microsporidia are obligatory intracellular pathogens; the only way to survive outside the host cell is in the spore stage (Vavra, 1976). The spores have a thick wall, which protects the sporoplasm (the infective germ) against unfavourable environmental conditions and makes it well suited for its function in transmission (Huger, 1961). Spores ingested by a host, germinate in the gut by extruding the so-called polar filament, which injects the sporoplasm into a host cell (Canning, 1971; Vavra, 1976). In the vegative phase of the life cycle, the germ undergoes several cellular divisions, and finally sporulation takes place (Vavra, 1976). The spores are released into the environment by excretion (with faeces or other excretion products), or when infected hosts die and desintegrate.

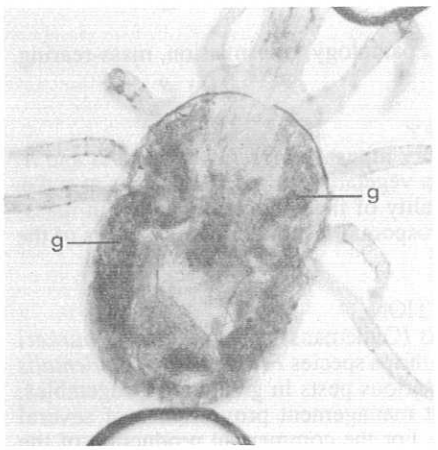

Figure 1: Disease-free A. barkeri *under light microscope, magn. 1270 x; g: gut.*

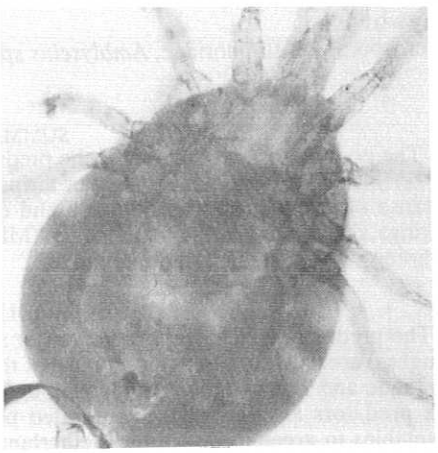

Figure 2: A.barkeri *with microsporidiosis under light microscope, magn. 1270 x.*

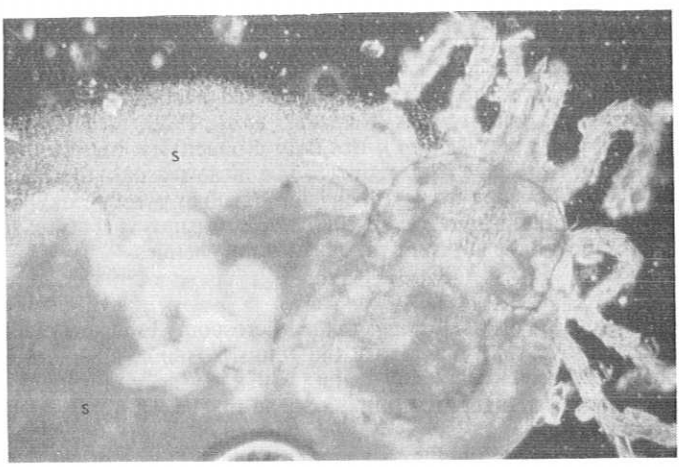

Figure 3: Squash preparation of A. barkeri *with microsporidiosis (same individual as in fig. 2) under phase-contrast light micrsocope, magn. 1270 x; s: spores.*

PATHOLOGY

Microsporidiosis in the predatory mites *A. cucumeris* and *A. barkeri* is only recognizable in heavily infected mites when the disease is in an advanced stage and when many spores have been produced. Such diseased mites have a swollen and whitish appearance and are sluggish in their movements. The symptoms are even more striking when the mites are observed under the light microscope: several organs (e.g., gut; see figure 1) are easily recognized in disease-free individuals, while internal structures in infected mites are hardly visible (figure 2). Squash preparations of mites in an advanced stage of the disease show numerous spores which have been formed in the cells of the mites, leaving hardly any healthy tissue fragments behind (figure 3).

In the mass-rearing, also infected prey mites have been observed. The symptoms of microsporidiosis in these mites are comparable to those in the predatory mites, although somewhat less conspicuous. Disease-free flour mites (and the other stored product mites) already have a whitish appearance and are slower in their movements than predatory mites.

QUESTIONS

In order to prevent future infestations in mass-rearings of predatory mites with microsporidia (or to cure already infected ones), it is important to know how the microsporidium persists in the culture. In other words: (i) Which conditions make it possible for the spores to survive outside the host? and (ii) How do mites become infected (or: how is the microsporidium transmitted)? The importance of the first question with respect to persistence of the disease in culture, depends on the number of free spores and on their contribution to transmission of the disease.

PERSISTENCE OF THE SPORES

Spores excreted (with faeces or other excretion products) by diseased mites, together with spores released after death of their hosts, form the "free-spore pool". On one hand, the pool increases in size by accumulation of spores; on the other hand it decreases since spores become inactivated by several factors. For many microsporidia ultraviolet light is one of the more destructive environmental factors (Ignoffo *et al.*, 1977; Kaya, 1975). In mass-rearings, spores are not directly exposed to sunlight, which means that UV-light does not play a prominent rôle in the inactivation of the spores. Temperature and relative humidity, on the other hand, may be important factors for survival of the free spores in mass-rearings. With increasing temperature and relative humidity, reduced spore viability has been observed (Gardner *et al.*, 1977), but tests on the persistence of spores under stored conditions, often vary with species of microsporidium: some microsporidia cannot even survive short periods of desiccation, while others require dry storage to prevent germination (Canning, 1982).

It is not known how the mass-rearing conditions (relative humidity ~90%; temperature ~22 °C) affect the virulence of the spores. Also other conditions of the mass-rearings (e.g., CO_2 concentration) may be of importance for the longevity of the spores. In addition, conditions that are negative for spore persistence, may well be positive for the development of microsporidia in mites, and vice versa. For example, a high temperature might reduce the virulence of spores, and at the same time it might accelerate the development of the microsporidiosis in the host.

TRANSMISSION OF THE MICROSPORIDIUM

Since the microsporidium occurs at two trophic levels (the predator and the prey), the possible ways by which the microsporidium may be transmitted are more extended than in case of a restriction to one host species. In our opinion, five possible ways of transmission of microsporidia may be distinguished. In addition to vertical transmission (from parent(s) to offspring) (v), four ways of horizontal transmission (h) may occur: by predation (h1), by contact with the free-spore pool (h2), by contact with the other

species or with conspecifics (other than feeding or mating) (h3), and by mating (h4) (see figure 4).

Since in the rearings not only infected predators but also infected flour mites (or other stored product mites) can be found, a possible way of transmission is ingestion of the spores when predatory mites are feeding on infected flour mites (h1a). Predatory mites are known to be cannibalistic, which means that the infection may be established by consuming diseased conspecifics as well (h1b). It seems less likely that flour mites become infected by diseased predatory mites; however, prey sometimes manages to escape from a predatory mite that started feeding on it (pers. obs. E.B.), and this may be just sufficient to transmit the microsporidium to the flour mite (h1a).

Contact with the free-spore pool may be an important way of transmission (h2), since the wheat bran and the fungi are contaminated by spores. By eating, or even tasting, all kinds of substances (like fungi), flour mites, and also predatory mites, may ingest spores. Even by cleaning their legs or mouthparts, infection might occur.

A male may tranfer spores along with sperm during mating, or might obtain spores from a diseased female (h4). Many microsporidia are transmitted to the offspring of the host by means of vegetative phases (v) (Canning, 1971), and this may well be the case with the microsporidium in the mass-rearing. The host may be either or both of the partents.

Physical contact with other mites (other than mating or feeding) (h3) may be considered as a fifth possibility, but one should realize that spores probably have to be ingested to become infective, and that it is not likely that diseased mites carry any other spores on the cuticula than those obtained from the free-spore pool (= transmission by contact with free-spore pool).

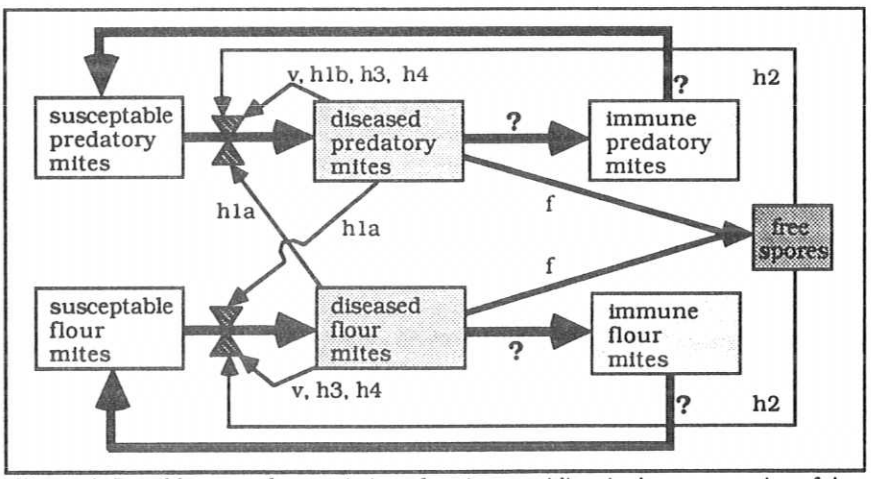

Figure 4: Possible ways of transmission of a microsporidium in the mass-rearing of the predatory mites Amblyseius cucumeris *and* A. barkeri *on the flour mite* Acarus siro *(or* Tyrophagus putrescentiae *and* Tyrolichus casei*).*

h1a: horizontal transmission by predation
h1b: horizontal transmission by cannibalism
h2: horizontal transmission by contact with free-spore pool
h3: horizontal transmission by contact with conspecifics or other species (not feeding or mating)
h4: horizontal transmission by mating
v: vertical transmission
f: contribution to free-spore pool by excretion by and death of diseased mites

Transmission probably depends on the incubation time and the infective dose. Once a host has been infected by a microsporidium, it takes time before spores are formed, before the infection has spread to the reproduction organs. Only then the microsporidium will be available for (horizontal or vertical) transmission. The rate at which the disease develops in a host, is probably set by the infective dose, *i.e.*, the number of spores that caused the infection.

In theory, it is possible the mites become immune to the microsporidium, especially when the infective dose is low. Once immune, they may become susceptible again. However, a memory component to the defence system, is thought to depend on life expectancy of animals. For short-lived organisms like mites, memory would be of limited value to their fitness (Anderson, 1986).

In case of transmission by predation (or cannibalism) the feeding behaviour of the mites has to be taken into account. Young predatory mites were found to eat only juvenile stages of flour mites or eggs, while the older predatory mites also preyed on the older ones (pers. obs. E.B.). These observations combined with the incubation time and infective dose dependency of the transmission, may very well restrict or even inhibit the transmission by predation.

CURRENT AND FUTURE RESEARCH

Currently we focus on sorting out which of the transmission mechanisms hold true. To do so, an efficient biological assay method is indispensable. Recently, Beerling (unpublished) developed such a method for predatory mites and flour mites. By means of a bioassay, several aspects of the microsporidiosis can be studied; isolated mites are fed known dosages of spores, and the effect can be followed in time. Changes in behaviour and appearence can be observed, and the development of the disease can be studied histologically by making sections at different moments after infection. The virulence of the micosporidium (LD_{50}, LT_{50}) can be tested for different mite stages and for different mite species.

This bioassay method may also be used to test spores which have been exposed to viability-reducing treatments (*e.g.*, high and low temperatures and relative humidities; chemicals), or to study the effect of different environmental conditions on the development of the microsporidium in the mites.

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SPECIFIC EPITOPES ON THE CAPSID OF POTATO LEAFROLL VIRUS MAY BE INVOLVED IN APHID TRANSMISSION

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Keywords:
Luteovirus, monoclonal antibody, *Myzus persicae*, virus transmission.

SUMMARY

Potato leafroll virus (PLRV) isolates which differed in their transmissibility by *Myzus persicae*, were tested in a triple antibody sandwich ELISA with a panel of nine monoclonal antibodies (MAbs). It was shown that four MAbs reacted significantly stronger with isolates which are readily transmitted than with the poorly transmitted ones. Furthermore, immunoblocking experiments in which suspensions of purified PLRV and MAbs were offered between Parafilm membranes for acquisition to *M. persicae*, revealed that these four MAbs considerably reduced the probability of virus transmission and significantly extended the latency period of the virus in its vector. The epitopes delineated by these MAbs might, therefore, be functionally involved in virus transport within the aphid.

INTRODUCTION

Potato leafroll virus (PLRV; luteovirus group), is a circulative virus, transmitted in a persistent manner by several aphid species of which *Myzus persicae* is the principal and most efficient one (Sylvester, 1980). During virus circulation through the vector's body, virus particles have to pass cell linings at gut and salivary gland level. A hypothetical model for transcellular transport of

luteoviruses at hindgut level was suggested by Gildow (1987). He observed that virus particles attached to the apical plasmalemma of the epithelium could induce endocytosis of the virus into a coated pit. These pits are directed towards the basal lamina of the cell where the particles are released into the heamocoel. In this model, the probability of a virus particle to be transcellularly transported is determined by its attachment to membranes by luteovirus-recognizing receptor molecules (Adam, Sander & Shepherd, 1979; Gildow, 1987). This may imply that protein structures on the viral capsid could play a key role in the passage of virus particles within their aphid vectors. In order to identify these structures, we compared readily and poorly transmissible PLRV isolates immunologically with a panel of monoclonal antibodies (MAbs) specific for PLRV. Moreover, we studied the question whether or not transmission of PLRV by *M. persicae* can be inhibited by blocking specific epitopes on the viral capsid with MAbs.

MATERIALS AND METHODS

Aphids. *M. persicae* biotype WMp2 was reared in cohorts differing one day in age on *Brassica napus* L. subsp. *oleifera* in a greenhouse at a 16-h photoperiod at 20±2°C (Van den Heuvel and Peters, 1989).

Virus. PLRV was maintained by repeated aphid transfer on *P. floridana* in a greenhouse at a 16-h photoperiod at 23±2°C. The PLRV-infected plants used in the experiments were infected in the cotyledon stage by single viruliferous *M. persicae* nymphs.

Antibodies. Nine monoclonal antibodies (MAbs) specific for PLRV, previously characterized by Van den Heuvel *et al* (1990), were used in a triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA).

ELISA. TAS-ELISA was done following Van den Heuvel *et al*, 1990).

Acquisition and transmission experiments. Mixtures of MAbs and purified PLRV were offered for acquisition to *M. persicae* nymphs via artificial diet MP148 (Harrewijn, 1983). After an acquisition access period (AAP) of one day the aphids were transferred to *P. floridana* seedlings for an inoculation access period. The probability of virus transmission and the median latency period (LP_{50}) were determined as described by (Van den Heuvel & Peters, 1990).

RESULTS

Immunological comparison. To study the relation between the transmissibility of PLRV and its surface structures, the reactivity of readily and poorly transmissible PLRV isolates was investigated in TAS-ELISA with the MAbs and mouse anti-PLRV PAbs. Six samples of leaf extracts from plants infected with the different isolates were tested. The ELISA values measured after 2 h at 405 nm (A_{405}) for each sample were related to the ELISA values obtained with mouse anti-PLRV of the same sample. The relative reactivity, expressed as the quotient of the A_{405} of the MAbs and mouse anti-PLRV, of PLRV isolates from top and bottom leaves of infected *P. floridana* plants (Van den Heuvel & Peters, 1990) are presented in Table 1.

Table 1. The relative reactivity (x100 %) ± S.E. of nine MAbs in TAS-ELISA with PLRV isolates from *P. floridana* .

PLRV isolates from aphid transmissibility		top leaves readily	bottom leaves poorly
MAbs	WAU-A2	11 ± 1	19 ± 2
	WAU-A5	21 ± 4	0 ± 0
	WAU-A6	41 ± 2	15 ± 1
	WAU-A7	42 ± 1	15 ± 1
	WAU-A12	125 ± 9	165 ± 6
	WAU-A13	18 ± 1	0 ± 0
	WAU-A24	134 ± 7	172 ± 14
	WAU-A47	89 ± 11	115 ± 10
	WAU-B9	52 ± 9	98 ± 10
Mouse anti-PLRV (PAbs)		100	100
LSD 5%		18	

PLRV from top leaves reacted significantly stronger with the MAbs WAU-A5, -A6, -A7 and -A13 than PLRV from bottom leaves. Also for the other comparisons made in TAS-ELISA between readily and poorly transmissible isolates it was consistently found that these four MAbs reacted stronger with the readily transmitted PLRV isolates.

Immunoblocking of PLRV transmission. The MAbs were mixed at different molecule ratios with purified PLRV suspensions of 60 µg per ml MP148. Rabbit anti-blackeye cowpea mosaic virus (BlCMV) antibodies which are not related to PLRV, served as a control in these experiments. The suspensions were offered for acquisition between Parafilm membranes to one-day old *M. persicae* nymphs during an AAP of one day. Afterwards, nymphs were transferred to *P. floridana* seedlings and the probability of PLRV transmission and the LP_{50} were determined in different experiments. Thirty nymphs were tested per experimental combination. The results obtained with a molecule ratio of 400 MAbs per virus particle are shown in Table 2.

Table 2. The inhibiting effect of simultaneous acquisition of MAbs and purified PLRV by *M. persicae* on virus transmission.

MAbs	% inhibition of transmission	LP_{50} (95% fiducial limits) h
WAU-A2	7	35 (29 - 40)
WAU-A5	32	45 (40 - 49)
WAU-A6	30	49 (45 - 55)
WAU-A7	23	50 (47 - 53)
WAU-A12	3	34 (29 - 37)
WAU-A13	30	50 (46 - 53)
WAU-A24	10	38 (32 - 42)
WAU-A47	27	41 (36 - 45)
WAU-B9	13	43 (37 - 48)
anti-BlCMV (PAbs)	0	35 (31 - 38)

The percentage of *M. persicae* nymphs that transmitted the virus after the simultaneous acquisition of MAbs and

PLRV was reduced by WAU-A5, -A6, -A7, -A13, -A47 and -B9). WAU-A2, -A12 and -A24 hardly had any inhibiting effect. Since virus particles might have precipitated after being mixed with antibodies and the percentage of transmission is known to be sensitive for the amount of virus acquired, also the LP_{50} was investigated (Table 2). This transmission parameter is not sensitive for the dose of virus acquired by the aphid (Van den Heuvel, Boerma & Peters, 1991). It was shown that only WAU-A5, -A6, -A7 and -A13 significantly extended the length of the LP_{50} as compared to the control treatment with anti-BlCMV PAbs.

DISCUSSION

PLRV isolates poorly transmitted by *M. persicae* were less readily detected by the MAbs WAU-A5, -A6, -A7 and -A13 than efficiently transmitted isolates (Table 1). Furthermore, these MAbs had an inhibiting effect on PLRV transmission when mixed with purified virus and offered to the aphids via artificial diets (Table 2). For these reasons we assume that the epitopes on the viral capsid delineated by these MAbs are functionally involved in virus transport within the vector. Competitive binding studies have revealed that WAU-A6 and -A7 are most likely directed against the same epitope, and that WAU-A5 and -A13 reacted with different but overlapping epitopes (Van den Heuvel *et al*, 1990). These MAbs all reacted with conformational dependent epitopes which are not sensitive for alkaline degradation and are supposedly formed by the tertiary protein structure of the viral capsid. In this aspect the epitopes they detect differ from the epitopes on the PLRV capsid associated with aphid-transmission as indicated by Massalski & Harrison (1987). They concluded that the poorly transmissible isolates lack one or more antigenic determinants presumably located on the quartenary protein structure. Also for geminiviruses which are transmitted by whiteflies and leafhoppers, it was suggested that the ability and specificity of circulative virus transmission is linked to

the antigenic specificity of the virus particle (Thomas, Massalski & Harrison, 1986; Harrison & Robinson, 1988).

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HOST SPECIFICITY AND OVIPOSITION BEHAVIOUR OF *TELENOMUS NITIDULUS*, EGG PARASITE OF THE SATIN MOTH, *LEUCOMA SALICIS*

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Keywords:
Monophagy, behaviour, *Telenomus nitidulus*.

INTRODUCTION

Poplar (*Populus* spp.) and willow (*Salix* spp.) are fast growing tree species, used in many countries for the production of wood. One of the most injurious insect pests affecting forests of these trees is the satin moth, *Leucoma salicis* (L.) (Lep.: Lymantriidae), which can cause large scale defoliation for periods of up to 4 years. Although the trees usually survive, outbreaks may cause a considerable reduction in growth (Luitjes, 1976). A potential biocontrol agent of the satin moth is the egg parasitoid *Telenomus nitidulus* (Hym.: Scelionidae). In several experiments, it was demonstrated that this parasitoid is host specific, univoltine and overwinters in the adult stage.

EXPERIMENTAL RESULTS

* Female adults of *T.nitidulus* which emerged in June and were stored in vials under the canopy of a forest stand from November to May of the following year, survived for an average of 370 days (survival percentage 87.5%) Maximum life span of one female parasite was 450 days. All male parasites died before the winter.

* Hibernating female adults were found overwintering in crevices and behind the bark of poplar trees in forest stands affected by a satin moth outbreak.

* The percentage parasitism of 90 egg masses collected in an outbreak area in July, varied between 0 and 100, with an average of 19 %. Although *T.nitidulus* is capable of digging tunnels through the spumilin cover of the egg mass, host eggs on the edges of the egg mass which remain uncovered, are

more frequently parasitized. The average percentage parasitism of the uncovered parts was 79 %, whereas that of the uncovered parts was only 11 %.

* In the literature, *T.nitidulus* has been mentioned as an egg parasite of *Agrotis segetum (Den. & Schif) (Lep.: Noctuidae)* (Kozlov & Kononova, 1983) and *Mamestra brassicae* (L.) (Lep.: Noctuidae) (Birova, 1979). In our trials however, eggs of *M.brassicae* remained unparasitized,when offered to the parasitoids. In addition, the size of the eggs of *M.brassicae* appear too small to harbour *T.nitidulus*. With respect to *A.segetum*, it seems unlikely that the parasitoid would overwinter in eggs of *A. segetum* if it is capable to overwinter without any difficulty in the adult stage. Moreover, in our experiments, *T.nitidulus* could not survive in the egg, larval, pupal or adult stage inside eggs of its host, during winter.

* Under laboratory conditions, *T.nitidulus* parasitized host eggs of up to 9 days old, but parasitization of 1-3 days old eggs was most successful. The average number of host eggs parasitized per female amounted to 202 (range 2-319). In the studies, the eggs were offered in an upside-down position, i.e. unprotected, with the spumilin layer underneath.

* Female parasites demonstrated a particular parasitization sequence consisting of: drumming with the antennae on the host egg, adoption of drilling posture, drilling, pumping, entering and removal of ovipositor and marking of the parasitized egg. If this parasitization sequence was broken off before marking, superparasitization occurred in 70 percent of the eggs, whereas marked eggs were only superparasitized in 5 percent of the cases.

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A NEW WINDTUNNEL FOR STUDIES ON HOST-SEEKING BEHAVIOUR OF MOSQUITOES

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Keywords:
Mosquito, *Anopheles gambiae*, host-seeking, behaviour, windtunnel, odour plume, method.

A new windtunnel was designed for the study of host-seeking behaviour of the malaria mosquito *Anopheles gambiae*. The study required that mosquito behaviour could be investigated at a constant temperature, with as little as possible effect of emanations from the observer, under night-time conditions and with adequate room for the mosquitoes to fly unhindered by walls and ceilings within and outside odour plumes. Outside air was filtered over glasswool and activated charcoal, passed through a temperature controlled heating device and led into a windtunnel. This was built of transparent polyacrylate with internal dimensions of 2x0.60x0.58m and the open ends were covered with copper gauze. Two ports were installed in one of the side walls for handling purposes. Wind speed and air temperature could be adjusted as required. Odour was released at the upwind tunnel section from a point source, at a speed similar to the tunnel air flow. Mosquitoes were released singly via remote control from a petri-dish placed at 1.20m downwind from the odour source. Because *An. gambiae* is a night-flier, all experiments were conducted under low light intensity conditions (5 lux, tungsten light bulbs). Mosquito behaviour was recorded with two CCD infrared-sensitive video cameras, connected to VHS recorders.

From a first series of experiments, in which the effect of human emanations on mosquito behaviour was studied, we obtained good results. Tunnel airflow was laminar, temperature remained constant at 25°C (when outside air temperatures fluctuated between 0 and 22°C) and mosquitoes flew through the tunnel without obvious signs of hindrance because of space limitations. Although all mosquitoes which responded flew initially inside the odour plume, because that is where they had been released, the majority flew upwind but outside the plume. Clear behavioural differences were observed between clean moist air, carbon dioxide (5%), human expired air and human skin emanations. Detailed results will be published elsewhere (Takken *et al.*, in prep).

Takken, W., De Bruyne M., Geervliet J.B.F. & Süverkropp B. (in prep) Behavioural responses of *Anopheles gambiae s.s.* Giles to carbon dioxide, human breath and skin emanations. - to be submitted to *Physiological Entomology*.

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DOES HAPLODIPLOIDY EXPLAIN REDUCED LEVELS OF GENETIC VARIABILITY IN HYMENOPTERA?

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Keywords:
Allozymes, bottlenecks, inbreeding, sociality, parasites, homeostasis.

Summary

Hymenoptera typically harbour low levels of genetic (enzyme) variability. A number of explanations for this result have been proposed, viz., haplodiploidy, environmental and behavioural characteristics, and genetic bottlenecks. More data, especially from the less advanced Hymenoptera as well as from other insect orders with haplodiploid species, are required to answer the question if haplodiploidy *per se* sufficiently explains the general dearth of genetic variation.

INTRODUCTION

Rapidly accumulating information from the last 25 years or so shows that many species harbour high levels of genetic variation at the enzyme level (Nevo et al., 1984). There is, however, much heterogeneity in variability levels among taxonomic groups as well as among species within taxonomic groups (Nevo et al., 1984; Menken, 1987). Hymenoptera, for example, display only about one-third the level of heterozygosity (H) of diploid-diploid insects (Berkelhamer, 1983; Graur, 1985; Unruh et al., 1986); sawflies may be an exception to this rule (Sheppard & Heydon, 1986; Woods & Guttman, 1987). Although there is widespread agreement that, compared to other insects, Hymenoptera are genetically depauperate, much speculation has arisen concerning the causes of this lack in overall variability. The following potential causes have been proposed: the haplodiploid genetic system, environmental and behavioural characteristics, and bottlenecks.

Enzyme number and choice strongly influence calculations of H levels (single locus heterozygosities differ much among loci [Powell, 1975; Simon & Archie, 1985]) and are thus confounding factors in species comparisons. The examination of a large number of

loci rather than a large number of individuals per locus leads to more reliable estimations of H (Nei & Roychoudhury, 1974; Archie, 1985). However, a negative correlation generally exists between the number of loci surveyed and the magnitude of H, as studies often start with known polymorphic loci (Singh & Rhomberg, 1987).

Allozyme electrophoresis can profitably be applied to a multitude of problems in agricultural entomology (for a review see Menken & Ulenberg, 1987). These include identification of pests and natural enemies, assessment of relationships among populations, their variability levels, patterns and magnitude of gene flow among populations and the like (Menken, 1990). Low levels of genetic variation might hamper much of such research.

RESULTS AND DISCUSSION

The following three major explanations for low levels of genetic variability in Hymenoptera have been proposed:

1. Haplodiploidy

Haplodiploidy can, for a variety of reasons, lower the potential for genetic diversity. These include: a] increased selection against slightly deleterious alleles, as all alleles are exposed in the hemizygous haploid males (Crozier, 1970); b] balanced polymorphisms are more difficult to be obtained in haplodiploid (or sex-linked) genetic systems (Mandel, 1959); c] reduced effective population size increases the effects of genetic drift and thus decreases genic diversity (Lester & Selander, 1979; Owen, 1985); d] increased levels of genetic linkage and hitchhiking owing to absence of recombination in males leading to a higher fixation rate of neutral alleles (Lester & Selander, 1979).

2. Sociality

Eusocial Hymenoptera live together in colonies that are organized around one to a few reproductives and thus have an effective population size that is usually a small fraction of the total adult population (Pamilo & Crozier, 1981), thus leading to a reduced level of genetic variation. Several aspects of the behaviour and the physical structure of nests might buffer environmental variability which, according to the niche-variation hypothesis (Van Valen, 1965) can lead to lowered genetic diversity. Endoparasitoids might also experience environmental homeiostasis. Present evidence, however, is inconclusive concerning the role of variable environments in maintaining a great deal of genetic diversity (Hedrick, 1986).

3. Bottlenecks

Actual levels of genetic variation in natural populations are often more related to time

elapsed since the last bottleneck, as well as to the duration and size of the bottleneck than to present day population sizes. Effects of genetic bottlenecks are believed to persist over many generations (Nei et al., 1975). Especially parasitic insects often face periodic bottlenecks (Lester & Selander, 1979). In addition, periods of small population sizes confer considerable potential for inbreeding (Berkelhamer, 1983; Woods & Guttman, 1987).

A selection of heterozygosity levels of hymenopteran species is presented in Table 1. The generally accepted conclusion that Hymenoptera as a group are genetically depauperate is based on a phylogenetically biased species sample (Sheppard & Heydon, 1986). A majority of the species studied belongs to only four superfamilies and many of these species are eusocial. Another group comprises endoparasitoids of the family Ichneumonidae (Menken, 1982). All these groups may be predisposed towards reduced levels of genetic diversity (sociality, inbreeding, and/or environmental homeostasis; Snyder, 1974; Lester and Selander, 1979). Furthermore, most solitary species studied are sawflies (see below), whereas most primitively and advanced eusocial species are bees and ants, respectively. To get to an unbiased estimate of levels of genetic variation in haplodiploids, more information is required from Hymenoptera with differing

Table 1. Estimates of genetic (allozyme) variation in Hymenoptera. Data from various sources (see Berkelhamer, 1983; Graur, 1985; Sheppard & Heydon, 1986; Unruh et al., 1987; Woods & Guttman, 1987; Packer & Owen, 1989 and 1990)

Family	Species	Heterozygosity	No. of loci
Diprionidae	*Diprion similis*	0.032	13
	Neodiprion taedae	0.026	15
	Neodiprion pratti-a	0.081	19
Tenthredinidae	*Pontania vesicator*	0.021	18
	Euura s-nodus	0.137	17
	Euura n.sp.	0.124	17
Argidae	*Schizocerella pilicornis*	0.167	16
Scoliidae	*Scolia dubia*	0.051	15
Andrenidae	*Andrena clarkella*	0.037	13
	Savastra obliqua	0.038	15
Apidae	*Apis mellifera*	0.012	16
	Bombus lucorum	0.010	16
	Bombus terrestris	0.037	15

	(Megabombus) lapidarius	0.007	16
Halictidae	*Augochlora pura*	0.000	24
	Augochlorella striata	0.107	47
	Halictus rubicundus	0.038	48
	Lasioglossum zephyrum	0.066	34
	Nomia heteropoda	0.070	15
Megachilidae	*Megachile pacifica*	0.033	17
Melittidae	*Macropis labiata*	0.033	10
Trigonalidae	*Trigona australis*	0.000	20
Vespidae	*Vespula vulgaris*	0.000	13
Sphecidae	*Mimesa equestris*	0.000	10
	Trypargilum politum	0.059	19
	Polistes annularis	0.053	15
	Polistes metricus	0.065	20
Formicidae	*Formica polyctena*	0.020	14
	Formica impressa	0.053	22
	Formica purpurea	0.005	22
	Iridomyrmex purpurens	0.039	15
	Nothomyecia macrops	0.032	16
Aphidiidae	*Aphidius ervi*	0.106	16
	Aphidius nr. *smithi*	0.008	16
Braconidae	*Opius juglandis*	0.043	13
Ichneumonidae	*Diadegma armillata*	0.048	19
	Pimpla turionella	0.038	29
	Triclistus yponomeutae	0.020	20
	Trieces tricarinatus	0.000	14
Encyrtidae	*Ageniaspis fuscicollis*	0.019	25
Eulophidae	*Tetrastichus evonymellae*	0.055	20

degrees of social organisation, nonsocial outbreeding species in particular. It is highly recommendable to screen biologically well-known species randomly sampled from all sociality groups.

Sawflies and hornflies (suborder Symphyta) are solitary Hymenoptera, the larvae of which exhibit varying levels of gregariousness (Smith, 1979). They are predominantly outbreeding, have potentially large effective population sizes, and females normally mate only once (Benjamin, 1955). Low genic variability must then mainly be attributed to haplodiploidy. The results of genetic screening in Symphyta are ambiguous (Table 1).

Sheppard and Heydon (1986) found two Tenthredinidae and one Argidae to be much more variable than other hymenopterans, similar to the average value for diploid insects. Woods and Guttman (1987), on the other hand, found reduced levels in *Neodiprion* much in accord with other Hymenoptera. One reason for this may be periodic bottlenecks.

Packer & Owen (1990), studying the extremely primitive halictid bee *Augochlorella striata*, arrive at an estimate of H = 0.107, the highest value found sofar for a bee species. Again, from a demographic point of view, this species is expected to experience frequent genetic bottlenecks. However, nest conditions and simple social behaviour make it likely that these bees experience a markedly fluctuating environment, possibly promoting a high H level (Packer et al., 1989).

Misinterpretations in the past have obscured the issue. Although Berkelhamer (1983) suggested that solitary and advanced eusocial Hymenoptera have significantly more variation than primitively social species owing to inbreeding, correct classification of the species as to their social level removed this difference (Graur,1985; Owen, 1985). The lowest level of genetic variability is found in eusocial species ($H = 0.028 \pm 0.005$; $n = 14$), solitary species have almost twice as much variation ($H = 0.058 \pm 0.008$; $n = 34$), and primitively eusocial species take an intermediate position ($H = 0.048 \pm 0.10$; $n = 11$; Packer & Owen, 1990 and references therein). In another case, a comparison between Heteroptera and solitary Hymenoptera leaded to the conclusion that "haplodiploidy *per se* does not reduce genetic variability" (Graur, 1985). After exclusion of data from heteropteran laboratory populations, which are likely to harbour less variability than the natural populations they were sampled from, this inference appeared to be unwarranted (Sheppard & Heydon, 1986).

Comparisons between haplodiploid and diplodiploid species within the same insect order (e.g., thrips, whiteflies, and mites) will provide the necessary data to test the hypothesis that haplodiploidy *per se* reduces genetic variation. Preliminary results (thrips, mites) indicate that such species indeed have low heterozygosity levels.

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KAIROMONE PERCEPTION BY A PREDATORY MITE: BEHAVIOURAL ANALYSIS OF CHEMORECEPTOR-CARRYING EXTREMITIES

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Keywords:
Phytoseiidae, *Phytoseiulus persimilis*, kairomone, chemoreceptor-carrying extremities, behaviour.

Summary
The predatory mite *Phytoseiulus persimilis* uses volatile and non-volatile kairomones in prey location. Chemoreceptors which may be used in perception of these kairomones are located on the tarsi of the first legs (olfactory chemoreceptors) and on the pedipalps (contact chemoreceptors). A method for quantification of the behaviour of chemoreceptor-carrying extremities of a predatory mite is described. This bioassay yields data on the intensity of examination of the environment. Upon stimulation with volatile and non-volatile kairomones both chemoreceptor-carrying extremities were used more intensively than in a control situation. This was obvious from increased movement frequencies (both extremities), increased amplitude of first leg movements and a larger proportion of time during which the pedipalps drummed the walking substrate.
When individual components of the *volatile* kairomone were offered on the walking surface, behavioural effects were only observed for first leg movements, but not for pedipalp behaviour. This suggests that volatile kairomones are only perceived by chemoreceptors on the first legs, which needs substantiating with electrophysiological investigations.

INTRODUCTION

The response of arthropods towards infochemicals (*sensu* Dicke & Sabelis, 1988) has been studied intensively by ethological investigations of the animal's movement patterns (Nordlund et al., 1981; Bell & Cardé, 1984) and electrophysiological investigations at the chemoreceptor level (Mustaparta, 1984; Roelofs, 1984). Chemoreceptors are often located on extremities such as antennae and legs, the behavioural characteristics of which are frequently mentioned to stress their role in the perception of chemical information (e.g., Jackson & Ford, 1973; Sabelis & Van der Baan, 1983; Klijnstra, 1985; Städler, 1986). Quantification of the movements of chemoreceptor-carrying extremities may be used to obtain behavioural evidence for infochemical perception. This may supplement data obtained from electrophysiological investigations and ethological investigations of the animal's movement patterns. For instance, while electrophysiological data disclose transmission of signals from the chemoreceptor to the central nervous system, data on extremity movements relate to behavior involved in sampling the environment.

Predatory mites (Acarina: Phytoseiidae) use volatile and non-volatile kairomones in prey location (Sabelis & Dicke, 1985). The predatory mite *Phytoseiulus persimilis* Athias-Henriot responds to kairomones related to the phytophagous spider mite *Tetranychus urticae* Koch. A volatile kairomone is emitted from leaves infested by these herbivores, but not from isolated spider mites, their webbing or exuviae (Sabelis & Van de Baan, 1983; Sabelis et al., 1984a) and a non-volatile kairomone is present on the infested leaf surface and spider-mite webbing (Hislop & Prokopy, 1981).

In a morphological study of the extremities of *P.persimilis*, contact-chemoreceptors have been identified on the tips of the pedipalps and chemoreceptors for volatiles on the

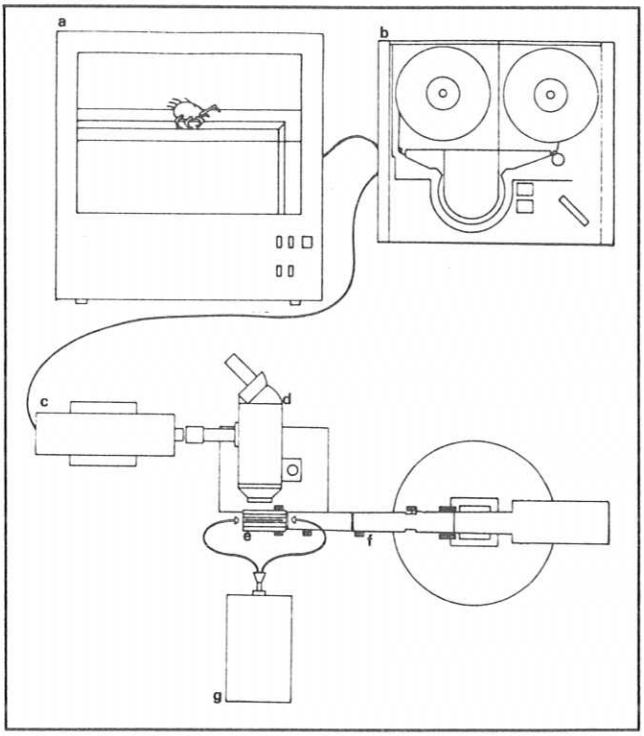

Figure 1:
Experimental setup. a: monitor; b: time-lapse video recorder; c: video camera; d: stereo microscope; e: microscrope slide/ 'moat on movable arm; f: movable arm; g: cold light source.

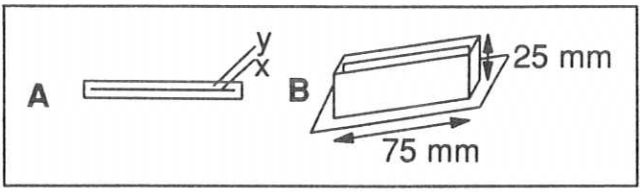

Figure 2:
Microscope slide / 'moat' combination. A: top view: x= microscope slide y= 'moat' B: side view.

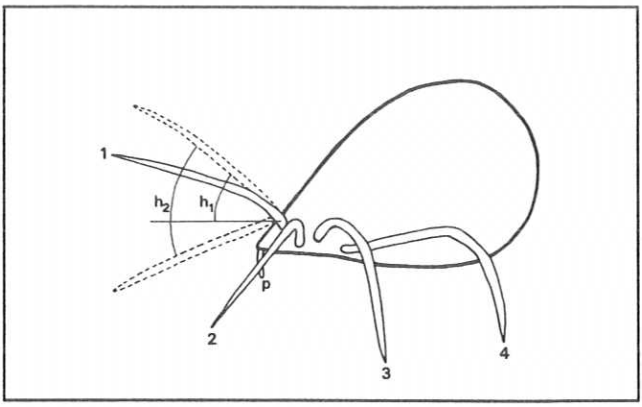

Figure 3:
Schematic sideview of predatory mite in which the different ways of quantifying height of first legs are indicated. h_1: angle between lowest and highest position of first leg; h_2: angle between highest position of first leg and horizon; p: pedipalp; numbers indicate legs.

dorsal side of the tarsi of the first legs (Jagers op Akkerhuis et al., 1985). These findings are in agreement with behavioural observations: pedipalps are used to drum individual prey, the intensity of which is correlated with prey acceptance (Jackson & Ford, 1973), and the first legs are not used in walking but are waved to and fro in the air (Sabelis & Van de Baan, 1983). This evidence on the location of chemoreceptors was used to develop a behavioural bioassay to quantify the use of these extremities in order to investigate what behaviour mediates perception of volatile and non-volatile kairomones by predatory mites.

MATERIAL AND METHODS

Mites *Tetranychus urticae* was reared on Lima bean plants (*Phaseolus lunatus* L.) at 20-30 °C, 50-70% r.h. under continuous fluorescent light that was added to the natural daylight regime.

Phytoseiulus persimilis was obtained from Koppert B.V. (Berkel en Rodenrijs, The Netherlands) and was reared at 20-30 °C on Lima bean leaves infested with *T.urticae*. Satiated predators were used in all experiments; they are known to respond to *T.urticae* kairomones (Sabelis & Van de Baan, 1983).

Bioassay Pedipalps and first legs of a predatory mite are moved vertically. Therefore, the best way to observe the behaviour of these extremities is to examine the mite from the side. Therefore, we observed predators laterally with a stereo microscope and videocamera, while it was walking on the side of a glass microscope slide (width 1 mm), surrounded by a 'moat' ca. 3 mm wide (Fig. 1 and 2). Thus, the predator could walk only along a glass 'alley' 7.5 cm long, and just a little wider than the mite itself. This ensured that the mite remains in focus. However, it may walk along the glass 'alley' and thus escape the view of the stereomicroscope/videocamera. To prevent this the microscope slide/'moat' combination is smoothly moved on rails to keep the mite's position in the centre of the field of view of the video.

A stimulus was applied onto the edge of the microscope slide. After introduction of the predator a videorecording was made for 15 min. This was analysed at low speed to quantify: (1) movement frequency of first legs; (2) movement frequency of pedipalps; (3) maximum height reached by first legs, expressed as the angle through which the legs are raised above the walking surface (see Fig. 3); (4) percentage of walking time during which pedipalps were drumming the surface; (5) percentage time spent preening the first legs in between the pedipalps; in doing so chemicals obtained from the environment may be exchanged by these extremities, and thus information may be shared; (6) frequency of stops and (7) walking speed, excluding time spent sitting still.

To apply stimuli onto the side of the microscope slide different substrates were lightly wiped over it: (1) an infested Lima bean leaf, freed of spider mites and their visible products; thus, both volatile and non-volatile kairomones were applied; (2) an uninfested Lima bean leaf; (3) a filter paper on which 5 µg of a synthetic chemical had been pipetted. The chemical was applied as a droplet to a small area of filter paper, which was then wiped over the microscope slide. The synthetic chemicals used were two kairomone components: linalool (Aldrich) and methyl salicylate (Aldrich) and a non-kairomone component: (Z)-3-hexen-1-yl acetate (Roth), which is also emitted from spider-mite infested leaves (Dicke et al., 1990); the 5 µg quantity, of which only a small, though unknown, fraction was actually applied on the glass, was based on data on release rates of these chemicals from spider-mite infested Lima bean plants (Dicke et al., 1990); (4) a clean filter paper. Before introduction of a predator, which occurred within a few minutes after application of the stimulus, the microscope slide was observed under a stereo microscope and any visible particle was removed from the walking surface.

Individual predators were used once only, each on a newly prepared slide. Experimental conditions were: 22-25°C and 60-80% r.h.

RESULTS

To determine which behavioural components change significantly in response to all relevant stimuli together, an initial experiment was set up in which 7 behavioural

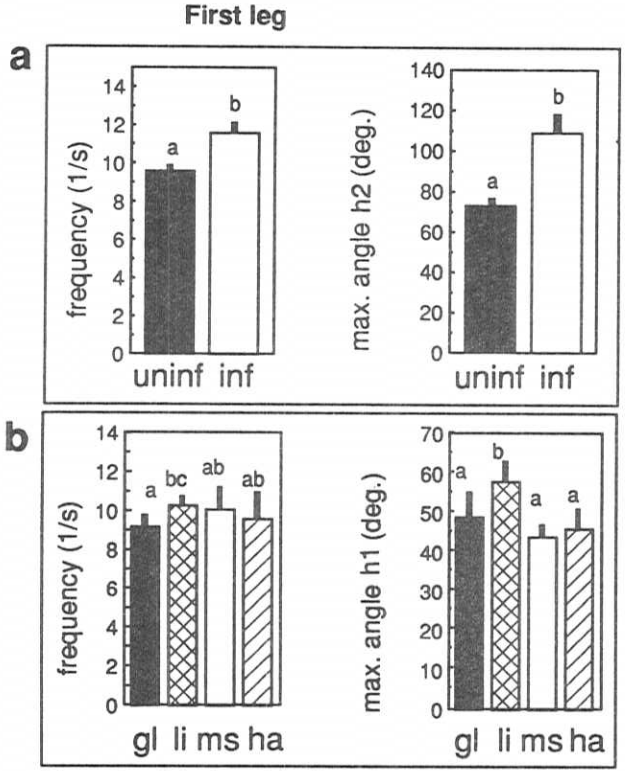

Figure 4: *Behaviour of first legs when (a) leaf chemicals or (b) synthetic chemicals are applied to the walking surface*
For explanation of max angle h1 and h2 see Fig. 2

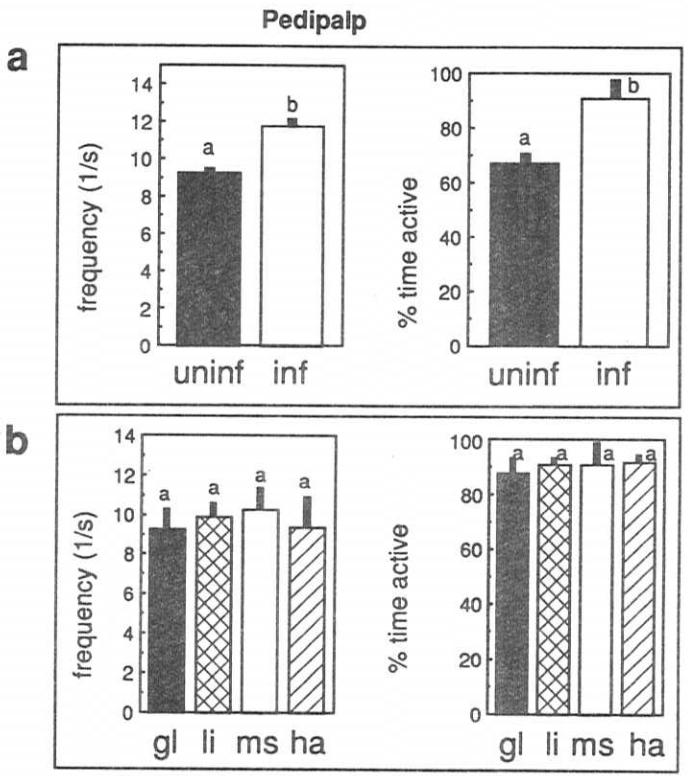

Figure 5: *Behaviour of pedipalps when (a) leaf chemicals or (b) synthetic chemicals are applied to the walking surface*

uninf= uninfested leaf (n=10); inf= infested leaf(n=10); gl= clean glass (n=20); li=linalool (n=10); ms=methyl salicylate (n=10); ha= (Z)-3-hexenyl acetate (n=8); bars within a graph that are indicated with different letters are significantly different (p=0.05; Mann-Whitney U test in Fig 3a and 4a; Kruskal-Wallis Multiple Comparison test in Fig 3b and 4b). The graphs give mean value and standard deviation.

parameters were quantified upon stimulation with chemicals obtained from infested or clean Lima bean leaves. The predators had a higher movement frequency of both extremities, moved the first legs higher above the substrate and were drumming with the pedipalps during a larger proportion of walking time upon stimulation with chemicals from infested leaves (including both volatile and non-volatile kairomones) when compared to stimulation with chemicals from uninfested leaves (Fig. 4a and 5a). Thus, behavioural changes occur both in extremities with olfactory chemoreceptors and those with contact-chemoreceptors. No significant differences have been observed in the other parameters (data not shown). Therefore, only the above four parameters were considered in subsequent experiments with individual chemicals.

Linalool elicits a behavioural change in first-leg behaviour which is similar to that observed after stimulation with natural kairomone, whereas methyl salicylate does not (Fig. 4b). Pedipalp behaviour was not affected by these chemicals (Fig. 5b).

No significant changes in extremity behaviour have been found after stimulation with (Z)-3-hexen-1-yl acetate (Fig. 4b and 5b), which is not a kairomone component (Dicke et al., 1990).

DISCUSSION

The bioassay described here elucidates differences in behaviour of chemoreceptor-carrying extremities in the presence or absence of kairomones. When volatile and non-volatile kairomones are applied, both extremities are used more intensively than in a control situation. Thus, the effect of kairomones on extremity behaviour affects chemoreceptor exposure to infochemicals. Blaney et al. (1986) mention that sensory input to the central nervous system may be modulated by changes in accessibility of the receptors, caused by e.g. closure of chemoreceptor-carrying orifices. In that context our observations on changes in extremity behaviour may be regarded as a behavioural change affecting sensory input to the central nervous system.

No intensified use of first legs or pedipalps has been observed upon stimulation with the kairomone component methyl salicylate or the non-kairomone component (Z)-3-hexenyl acetate. Thus, current data parallel results on the behavioural response in a Y-tube olfactometer for linalool and (Z)-3-hexenyl acetate, but this does not seem to be so for methyl salicylate (Dicke et al. 1990). Linalool affected the behavioural response of *P. persimilis* at much lower dosages than methyl salicylate (Dicke et al. 1990). Thus, the amounts of methyl salicylate applied in our current experiments may have been too low to elicit a behavioural response in predator extremity behaviour.

The above considerations imply that conclusions from olfactometer experiments and current data on extremity movements parallel for at least two of the three chemicals tested. However, it is not expected that this will generally be the case. The bioassay developed here provides data on just one event in the process of information conveyance through chemicals: moving of chemoreceptor-carrying extremities and thus affecting chances of infochemical interception.

When a predator is walking on the substrate from which chemicals evaporate, it may perceive these chemicals through olfaction or taste. Distinction between these modes of perception through ethological investigations requires well-designed experiments (see Sabelis & Dicke, 1985 for discussion). Our data show that, compared to a control, the kairomone component linalool elicits a higher movement frequency and amplitude of first legs, which are equipped with olfactory chemoreceptors. No intensified use of pedipalps, equipped with contact chemoreceptors, was observed. This suggests that linalool is only perceived by olfactory chemoreceptors on the first legs but it is no proof of that: perception by contact chemoreceptors on the pedipalps might lead to intensified use of first legs and not of pedipalps. Experiments with the current bioassay in which stimulation is done with a distant linalool source may be a next step in elucidating which chemoreceptors perceive the volatile kairomone, but then adsorption of linalool to the walking substrate (cf. Wall et al. 1981; Wall & Perry, 1983) and subsequent tasting may still occur. Ultimately, electrophysiological investigations are needed to conclusively elucidate by which chemoreceptors the volatile kairomone component is perceived.

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OPTIMAL CLUTCH SIZE OF PARASITOIDS IN STOCHASTICALLY FLUCTUATING ENVIRONMENTS

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Keywords:
Optimal clutch size, parasitoid-host interaction, stochastic environment.

Summary

In parasitoids, clutch size affects the survival chances of the larvae as well as the sizes of emerging daughters, and thus the egg supplies of the next generation. In *Tetrastichus hagenowii*, the survival chance per larvae increases with clutch size, but the mean size of emerging offspring decreases. We studied the effects of these relations on the expected long term population growth rate in different environments, by means of population dynamic models. Whereas in deterministic environments the optimal clutch size is fixed, in environments that vary stochastically over generations variable clutch sizes are often optimal.

INTRODUCTION

Most parasitoids do not lay all the eggs they have at their disposal in one host, even if the host is big enough to support all eggs. Furthermore, even between individuals with identical egg supplies there are often large differences in actual clutch sizes. It can be expected that clutch size affects the survival chances of larvae as well as the sizes of emerging offspring. Size, in turn, will (at least partly) determine the egg supply of females. Thus, clutch sizes within one generation probably influence the clutch sizes of the next generation.

Relations between egg supply, clutch size and number and sizes of offspring were studied in *Tetrastichus hagenowii*, an oöthecal parasite of cockroaches. The main findings were:

1. Primary egg supply is correlated with body size
2. Clutch size increases with egg supply, but usually not all eggs are laid
3. The larger the clutch, the higher the survival chance per larva
4. Clutch size influences the size of the daughters: small clutches produce on average large daughters, large clutches give small daughters.

It can be expected that results (1) and (4) hold for most parasitoids. Relation (3) may be different in different species, depending on wether or not there is competition between larvae. In this case, apparently the ability to defeat a host's defense reaction increases with the number of larvae.

We studied the consequences of different clutch size distributions on the expected long-term population growth rate, by means of a population dynamic model. For different environments, varying stochastically over the generations, we

determined which strategy led to the maximal expected long-term growth rate. This is the optimal strategy, since it gives the largest expected number of offspring per individual in the long run. Relations (1), (3) and (4) were built into the model, to determine their effects on the optimal clutch size distribution. Note that, with respect to selection pressures on clutch sizes, relations (3) and (4) have opposing effects.

Description of the model

To get an impression of the effects of environmental factors as well as causal relations between clutch sizes and offspring, we considered a simplified situation with two types of females, large ones, with 2 eggs, and small ones, with only one egg. Furthermore, since in *Tetrastichus hagenowii* sex-ratios are extremely female-biased, we disregarded sex-ratio and considered an all-female population. In generation t, the chance that a large female survives until she meets a host is λ_t, and for small females this chance is μ_t. When a large female encounters a host, she can either lay 2 eggs or 1 egg. We assume that she lays 2 eggs with chance 1-p and 1 egg with chance p. (When p is 0 or 1 the strategy is deterministic, e.g. when p =0, always 2 eggs are laid.). To summarize, we have the following relations:

Large female:		Small female:
survives with chance λ_t		survives with chance μ_t
with chance p:	with chance 1-p:	
lays 1 egg	lays 2 eggs	lays 1 egg
offspring:	offspring:	offspring:
D_{11} small females	D_{12} small females	D_{11} small females
D_{21} large females	D_{22} large females	D_{21} large females

D_{ij} is the expected number of daughters of size i from a clutch of size j.

Let $N_1(t)$ be the number of small females in generation t and let $N_2(t)$ be the number of large females, then:

$$N_1(t+1) = \mu_t D_{11} N_1(t) + \lambda_t (p\, D_{11} + (1-p)\, D_{12}) N_2(t)$$
$$N_2(t+1) = \mu_t D_{21} N_1(t) + \lambda_t (p\, D_{21} + (1-p)\, D_{22}) N_2(t)$$

The survival chances $\mu_1,\ldots,\mu_t$ vary independently over the generations, with expectation E1 and variance σ_1^2. Similarly, the chances $\lambda_1,\ldots,\lambda_t$ are independent and identically distributed with expectation E2 and variance σ_2^2. Furthermore, for each i, μ_i and λ_i have correlation r.

The expected long-term growth rate of the population was approximated by means of a perturbation expansion (Tuljapurkar, 1990 and Caswell, 1989). The value of p leading to the largest expected long-term population growth rate is the optimal strategy. We studied the effects of the D_{ij}'s as well as the expectations and variances of survival chances on the optimal strategy p*.

Results and Discussion

For a given set of D_{ij}'s, the optimal strategy depends on the ratio E2/E1, the coefficients of variation of the survival chances, $c1(=\sigma_1/E1)$ and $c2(=\sigma_2/E2)$, and the correlation r. We only consider cases where small clutches give relatively many large daughters and vice versa. Thus, $D_{11}<D_{12}$ and $D_{21}>D_{22}$.

When both variances are zero, i.e. when the survival chances of females are fixed, the optimal strategy is to lay 1 egg when

$$E2/E1>\xi,$$

where

$$\xi = \frac{(D_{12} - D_{11})}{(D_{21} - D_{22})}$$

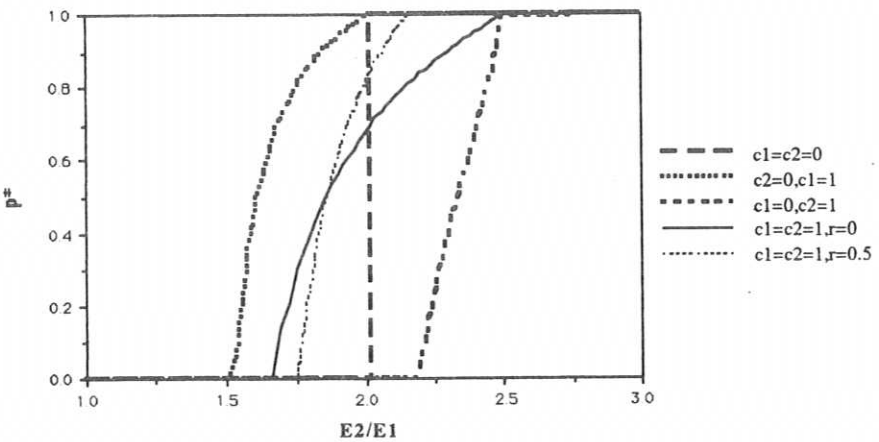
Note that, since large clutches produce more daughters, ξ is larger than 1. When the inequality is the other way round, 2 eggs should be laid. When E2/E1 is equal to ξ, the long-term population growth rate does not depend on clutch size (see figure). Thus, unless E2/E1 is equal to ξ, in this case the optimal strategy is fixed. Dependent on the environmental circumstances, females should either always lay one or always two eggs. Thus, we see that under some conditions it is indeed profitable only to lay one egg, even though there is a supply of 2 and no other hosts are encountered, namely when the expected gain in small daughters that survive until reproduction at a clutch size of 2 is smaller than the expected gain in large surviving daughters at clutch size 1. However, in such an environment one would expect evolution towards large females with a small egg supply, since the extra eggs are never used. Variable clutch size strategies could occur when E2/E1 is exactly equal to ξ. Here, too, one would expect all females to have small egg supplies in the long run,since obviously they would not need to put extra investment into a large egg supply under these circumstances.

When the survival chances λ_t and μ_t vary stochastically over the generations, we find variable optimal strategies, i.e. individuals with an egg supply of 2 should lay 1 or two eggs with a certain probability which is not equal to 1 or 0. This phenomenon, called 'bet hedging' (see Stearns, 1976) is found more often in stochastically varying environments. It can be explained as follows. Suppose that large females always lay two eggs. As a consequence, when in the current generation there are many small females, the next generation will contain many large ones and vice versa. However, when circumstances are coincidentally bad, and there are many small females, almost all offspring will die. Thus, to spread the risk, there should be a certain proportion of large daughters with a larger survival chance in each generation. On the other hand, when females would always lay 1 egg, regardless of their supply, they would not take optimal advantage of good environmental circumstances, when many small females survive.

The effects of stochastic fluctuations in survival chances are illustrated in the figure, for the case that $D_{11}=0.01$, $D_{12}=1.99$ $D_{21}=0.99$, $D_{22}=1.99$. Note that, in this example, all eggs hatch and all larvae survive, since $D_{11}+D_{21}=1$ and $D_{12}+D_{22}=2$. We will come back to the effects of differential survival of large clutches later.

In all cases, p* increases as the expected survival chance of large females relative to that of small females increases. This is intuitively clear, since the advantage of having large daughters increases with increasing E2/E1. When there

are large fluctuations in survival chance of small females, the risk of losing many small daughters when circumstances are bad increases. Thus, the optimal p* increases with increasing c1. On the other hand, an increase in coefficient of variation of the survival chance of large daughters decreases the advantage of large daughters, and therefore decreases p*.



Effects of expectationsof, variances in and correlations between survival chances on optimal chance of laying one egg when the supply is two. In the considered situation, ξ=2.02. Further explanation see text.

As a result, when both c1 and c2 are larger than zero, the optimal chance of layig one egg may be larger than zero for E2/E1<ξ and smaller than one for E2/E1>ξ. When the coefficients of variation c1 and c2 are equal, increase in correlations between λ_t and μ_t causes a slight decrease in p* (as compared to the uncorrelated case) when E2/E1 is relatively small and an increase when E2/E1 is high.

Besides the characteristics of fluctuations in survival chances of large and small daughters, the expected numbers of small and large daughters produced by different clutch sizes also affects the optimal p*. As can be expected, p* decreases when small clutches produce less large daughters and/or when large clutches give more large daughters, since in both cases the relative advantage of small clutches in terms of giving more large offspring decreases. Furthermore, the optimal p* decreases when the survival chance until emergence in small clutches is smaller than that in large clutches. However, even in that case there are circumstances in which it is advantageous to have a small clutch size.

To conclude, even when only one host is encountered, it may be advantageous not to lay all available eggs. One possible cause for this is that large females have a larger survival chance than small females. However, this is not the only possible explanation for small clutch sizes. In stochastically fluctuating environments, large variance in survival chances of small females may also lead to small clutch sizes. We even found that, when c1 is much larger than c2, it is optimal to lay small clutches when expected survival chance of large daughters is lower than that of small daughters. Furthermore, in stochastic environments, variable clutch size strategies are sometimes optimal. Thus, variation in survival chances of females over the generations may explain the often observed phenomenon of large inter-individual differences in clutch size

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COMPARISON OF TWO *COTESIA* SPECIES FORAGING FOR SOLITARILY AND GREGARIOUSLY FEEDING *PIERIS* HOST SPECIES

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Keywords:
Cotesia spp., Pieris spp.., parasitoid, foraging behaviour, searching strategy.

Summary

In a semi-field set-up we compared the foraging behaviour of *Cotesia rubecula*, a specialist parasitoid of solitarily feeding *Pieris rapae* larvae and *Cotesia glomerata*, a more polyphagous parasitoid that attacks several Pieridae species but mainly the gregariously feeding *Pieris brassicae*. The experimental set-up consisted of Brussels sprouts plants infested with one or the other host species or both. *C. rubecula* showed preferential behaviour towards *P. rapae* and its searching strategy seems adapted to foraging for solitarily feeding hosts. The polyphagous *C. glomerata* showed more plasticity in its foraging pattern. Although no preferential behaviour towards *P. brassicae* was observed in *C. glomerata*, females foraged more efficiently for *P. brassicae* than for *P. rapae* as their searching strategy seems more adapted to foraging for gregariously feeding hosts.

INTRODUCTION

Comparing phylogenetically related species that differ in some ecological characteristic is a valuable tool for inferring the adaptive value of species traits. This comparative approach, which is applied in the present study, has already shown its value for the functional interpretation of differences in foraging behaviour of parasitoids (e.g. Vet & van Alphen, 1985).

Two related *Cotesia spp.* (Hymenoptera: Braconidae) are natural enemies of *Pieris* larvae feeding on cruciferous plants such as *Brassica species*. The solitary *C. rubecula* is considered an obligate parasitoid of larvae of the solitarily feeding small cabbage white, *Pieris rapae*, but it is also attacks the large cabage white, *Pieris brassicae* (Shenefelt, 1972). The gregarious *C. glomerata* is a more polyphagous parasitoid of several Pieridae species among which *P. rapae*, but its major host species in Europe is *P. brassicae*, whose caterpillars feed gregariously (Laing & Levin, 1982). In the present study we investigated the searching behaviour and efficiency of the two parasitoid species foraging in a semi-field set-up of clean plants mixed with plants infested by either *P. brassicae, P. rapae*, or both. Absolute densities of host larvae in these set-ups were identical, but the hosts were distributed in their natural pattern, i.e. *P. brassicae* larvae were clustered and *P. rapae* larvae fed solitarily.

MATERIALS AND METHODS

Parasitoids The parasitoids used in the experiments were 4 to 7 days old *C. glomerata* and *C. rubecula* females. *C. glomerata* wasps were reared on *P. brassicae* larvae, while *C. rubecula* wasps were reared on *P. rapae* larvae. Both host larvae fed on Brussels sprouts plants (*Brassica oleracea* cv. Titurel). The cocoons of the wasps were collected in Petri dishes and stored at 24°C until emergence. They were then t-ransferred to a 15°C incubator. Three days later the females were put in a small jar with a piece of humid cotton and a drop of honey. Parasitoids were maintained at 15°C. Prior to an experiment females were individually transferred to a small vial.

Plants Infested plants were obtained by putting two month old uninfested Brussels sprouts plants (12 leaves) in an oviposition cage with butterflies. Following oviposition the plants were taken out and the number of eggs was reduced to 20 per plant. In the case of *P. brassicae* this resulted in 1-2 clusters per plant. For *P. rapae* 8-10 leaves per plant were infested with 1-4 eggs per leaf. Twenty-four hours after hatching of the eggs, the plants were used in the experiments. Plants were used for only one day. After each replicate parasitized larvae were removed and replaced by unparasitized ones.

Experimental procedure All experiments took place in a greenhouse compartment at a temperature of 24°C and a relative humidity of 60%. The light intensity (natural light only) fluctuated between 1000 and 8000 lux. In this compartment a "tent", made of white sheets, was constructed to obtain a contrasting background for the parasitoids. On a table eight plants were placed in two parallel rows. Four of these plants were clean, the other four were infested. Plant position was random. Two fans, placed behind a piece of netting at the end of the table, provided an airstream of 0.3 - 0.4 m/s at the release site. This release site, situated at the downwind end of the table, consisted of an excised Brussels sprouts leaf with feeding damage (no hosts), inflicted by the same host or hosts as present in the particular experiment. This leaf was put in a small jar filled with water.

The foraging behaviour of the parasitoids was observed continuously and recorded on a portable computer using the program "The Observer" (Noldus, 1990). An observation started at the moment a parasitoid left the release site and flew to one of the eight plants. Experiments were terminated after 1 hour or when the parasitoid left the foraging arena and remained on the tent screen for more than 1 minute.

The following parameters were recorded:
1. Behaviour of the parasitoid: *Fly; Walk:* the antennae are extendend in front of the head and are alternately moved up and down; *Search:* the antennae are bent and swept along the surface; *Attack*: the wasp draws her abdomen between her legs and raises her antennae and wings; *Oviposit*: the ovipositor is inserted into a host larva; *Stop:* the wasp is motionless except for antennal movement; *Groom:* the wasp cleans her antennae, wings and abdomen.
2. Plant number: the plants were numbered 1 to 8.
3. Leaf number: the leaves per plant were numbered 1 to 12 from bottom to top. Before an experiment each leaf was examined and the number of larvae was noted.

Three different experiments per parasitoid species were tested, whereby 8 plants consisted of:

Exp. I. Four plants infested with *P. brassicae* larvae and four clean plants;
Exp. II. Four plants infested with *P. rapae* larvae and four clean plants;
Exp. III. Two plants infested with *P. brassicae* larvae, two with *P. rapae* larvae and four clean plants.

RESULTS AND DISCUSSION

The behavioural experiments provided abundant data on the foraging behaviour of the two parasitoid species. Only part of these data is given in the present paper.

The maximum foraging time was set at one hour, but parasitoids did not always spend a full hour foraging in each experiment. Differences in this time may indicate differences in motivation of the parasitoids to search for and parasitize the different host species. Table 1 shows that the foraging times for *C. glomerata* do not differ between the three experiments *C. rubecula* however, stays longer in the arena with *P. rapae* as a host and shorter when *P. brassicae* alone is present in the set-up.

Table 1: Mean foraging time (in sec) of *Cotesia spp.* in the three experiments.

Parasitoid	Host species present	Foraging time (sec)[1]	n[2]
C. glomerata	*P. brassicae*	3600 [a]	20
	P. rapae	3251 [a]	18
	P. brassicae/rapae	3479 [a]	24
C. rubecula	*P. brassicae*	1303 [x]	23
	P. rapae	3138 [y]	18
	P. brassicae/rapae	2632 [y]	21

[1] Observation times with different letters are significantly different (Multiple comparisons test $p < 0.05$, based on Kruskal-Wallis rank sums).
[2] Number of females tested.

After taking off from the release site, females of both species flew significantly more to an infested plant than to a clean plant (3 experiments pooled, table 2). The third experiment (choice sitation) shows that *C. rubecula* significantly preferred to land on *P. rapae* infested plants compared to *P. brassicae* infested plants, while no significant preference was found for *C. glomerata*.

The host-finding and ovipositional behaviour of both parasitoids on the two different host species are summarized in ethograms (Figure 1a-d). The host-finding behaviour of *C. rubecula* on *P. rapae* (Fig. 1a) is similar to the host-finding behaviour of *C. glomerata* on that same host species (Fig. 1c). The main difference is that *C. rubecula* always moves away from the site of attack after an oviposition, either by walking or by flying (see also Nealis, 1986), whereas *C. glomerata* resumes searching in the same area in 20% of the cases (see also Sato, 1979).

Comparison of the two ethograms of *C. rubecula* shows that the host species does not influence her behaviour to a great extent (Fig. 1a-b). When foraging for *P. brassicae*, *C. rubecula* leaves the site of attack somewhat more by flying than by walking, which may be due to the more aggressive defense behaviour of *P. brassicae* larvae.

Table 2: Number of first landings on clean plants vs. infested plants (in all 3 experiments) and *P. brassicae* infested plants vs. *P. rapae* infested plants (in choice experiment)

C. glomerata	Clean vs. Infested			*P.brassicae* vs. *P.rapae*		
I. *P. brassicae* (only)	6	14				
II. *P. rapae* (only)	7	11				
III. *P. brassicae/rapae* (choice)	5	19	*	8	11	
Total	18	44	*			
C. rubecula	Clean vs. Infested			*P.brassicae* vs. *P.rapae*		
I. *P. brassicae* (only)	7	15				
II. *P. rapae* (only)	3	15	*			
III. *P. brassicae/rapae* (choice)	5	15	*	3	12	*
Total	15	45	*			

Asterisk indicates significant difference (Chi-square test $p < 0.05$).

The behaviour of *C. glomerata* is more plastic as they forage differently for the two host species (Fig. 1c-d). The presence of the gregariously feeding *P. brassicae* leads to a repeated cycle of attack and oviposition. Females remain at the site of attack. In 60% of the replicates 70-100% of the cluster was parasitized when she left. This was never observed for *C. rubecula*, as females of this species never displayed any area restricted search.

How do these different searching strategies affect the parasitization success in the different experiments? Large differences in foraging success were observed between the parasitoid species in the different experiments (Figure 2). *C. glomerata* foraged most successfully for the gregariously feeding *P. brassicae* larvae (Fig. 2a). During an equal period of time about 5 times as many larvae were parasitized in experiment I (= *P. brassicae*) as in experiment II (= *P. rapae*). In the choice-experiment this ratio did not significantly change.

C. rubecula, on the other hand, foraged most succesfully for *P. rapae*. About 5 times as many *P. rapae* larvae were parasitized in experiment II as *P. brassicae* in experiment I. In the choice situation this ratio of *P. rapae* to *P. brassicae* parasitized even increased to 22:1, which indicates a clear preference for *P. rapae*.

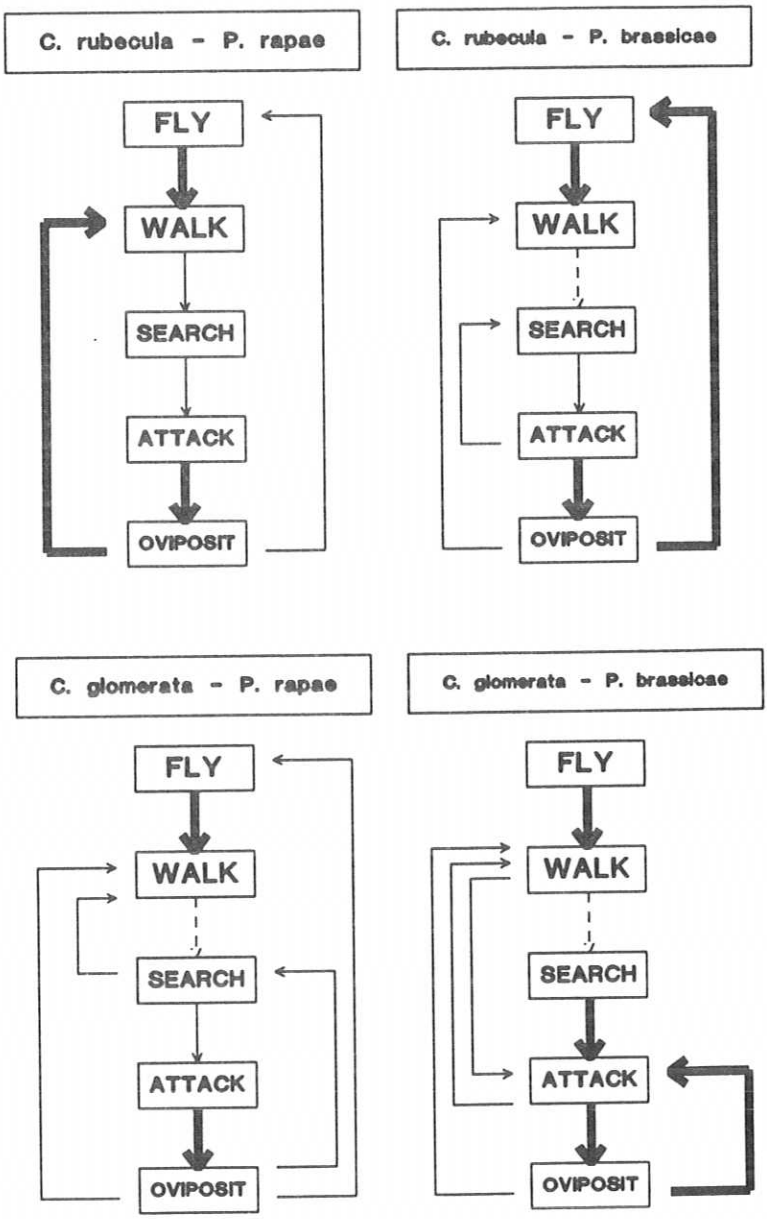

Figure 1. *Summarized ethograms of the foraging behaviour of C. glomerata and C. rubecula in a setup with either P. brassicae or P. rapae as a host (different arrows represent difference in frequency of one kind of behaviour following the other; thick arrows > 50%, thin arrows 20-50%, dashed arrows 10-20%).*

The fact that *C. rubecula* parasitized more *P. rapae* can partly be explained by active foraging decisions that incite preference. These foraging decisions are landing preferences and different time allocation to plants infested with different host species. In addition to this expressed preference, the low number of *P. brassicae* parasitized can be explained by the foraging strategy of *C. rubecula*, which seems adapted to solitarily feeding hosts. Whereas area restricted search would be functional in the case of the gregariously feeding *P. brassicae*, *C. rubecula* flies or walks away from the site of attack after an oviposition. For *C. glomerata* no foraging decisions, leading to preference, were observed. The fact that *C. glomerata* parasitized more *P. brassicae* can be fully explained by her searching strategy that, although being somewhat more plastic than that of *C. rubecula*, seems most adapted to foraging for gregariously feeding host larvae.

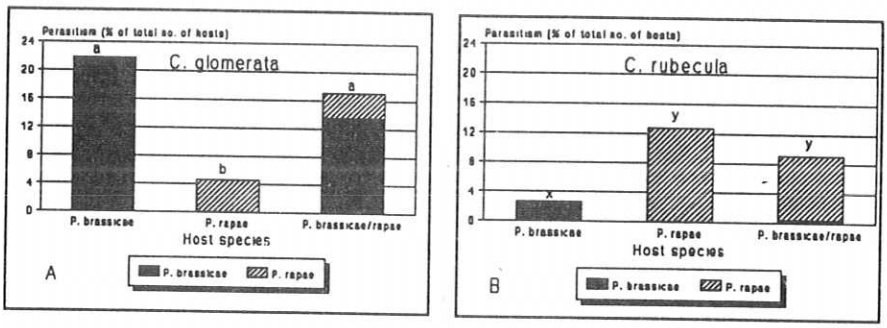

Figure 2. *Parasitism (% of the total no. of hosts) in the three different experiments (bars with different letters are significantly different; multiple comparisons test $p < 0.05$, based on Kruskal-Wallis rank sums).*

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AERIAL RELEASE OF ACARINE BIOLOGICAL CONTROL AGENTS ON CARRIER MATERIALS

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Keywords:
Aerial release, *Neoseiulus idaeus*, biological control, carrier materials.

SUMMARY

Laboratory and field experiments were performed to test several materials on their suitability as carrier for the aerial release of phytoseiid mites. Relatively few mites adhered to the materials. Nevertheless, mites were retrieved in cassava fields after a mass release, using oat flakes as carrier material. This method can be suitable when large areas are to be supplied with low mite densities.

INTRODUCTION

The cassava green mite (CGM), *Mononychellus tanajoa* (Bondar) and the cassava mealybug (*Phenacoccus manihoti* (Matile Ferrero)) are the most serious pests on cassava (*Manihot esculenta* (Crantz)) crops. Both pests were accidentally introduced in Africa via contaminated plant material from the neotropics (Herren & Bennett, 1986). Against the mealybug, an efficient biocontrol agent was found, and dispersed over the African cassava belt by means of an aircraft equipped with an Airborne Insect Release Sytem (AIRS), especially designed for this purpose (Herren *et al.*, 1987).

Efficient biocontrol agents against CGM are being identified by IITA's Biological Control Program and are likely to be available in mass production in the near future (Friese *et al.*, 1987, Mégevand *et al.*, 1987, Haug & Mégevand, 1989). In view of this, methods of dispersing these biocontrol agents need to be developed.

Previous research (Pickett *et al.*,1987) suggests that phytoseiids can be released aerially. Because of the availability of the AIRS, developed for releasing the mealybug parasitoid *Epidinocarsis lopezi* (De Santis), this study elaborates upon this method. Since the biocontrol agents against CGM, phytoseiid mites, differ in many respects from *E. lopezi*, the way in which these mites are released needs modification. Phytoseiids are smaller in size and have therefore different aerodynamic properties. They will behave more like aerosols and should float on air for some time before coming down. For this reason the place of landing may not be near the place of release (Bird, 1986, Johnson & Croft, 1981). Phytoseiids have no wings and are passively transported by air currents although they can determine the moment of taking off (Johnson & Croft, 1976). Because of this passive way of transportation, it is important that a release includes active transport to the plant.

Three ways of packing biocontrol agents for aerial release were taken into consideration. First, by inserting agents without any material added in the release cassette. Second, by adding a carrier material to which the agents can adhere upon during precipitation. Third, by packing agents in a closed container, which opens at release.

The first method, used previously for insects is, however, not suitable for mites

because of their tendency to stay on the walls of the release tubes and because of their inconspicuousness which, in combination with a larger downwind drift, will make sampling extremely difficult (Bird 1986).

Drukker *et al.* (manuscript) designed and tested a package that opens automatically at release and lodges on the cassava plant canopy thanks to a sophisticated container-wire-counterweight construction. They checked mortality and fecundity of the mites and tested the package at several fly-over altitudes. This method does not allow high quantities of mites to be released in a field when flying over only once. For inundative releases, larger numbers of mites per shot will be necessary. The use of carrier material instead of a container might be a way of releasing more mites in one shot.

Carrier material for aerial releases of phytoseiids was first used by Pickett *et al.* (1987). Their phytoseiids were mixed with corncob grits, stored and spread by a 'mechanized, refrigerated delivery system' (Pickett *et al.*, 1990) which was housed in the aircraft. The phytoseiids released were recovered in the fields, although in low densities.

In this study properties of some carrier materials are evaluated for use in aerial releases with the AIRS.

MATERIALS AND METHODS

The aircraft and the Airborne Insect Release System (AIRS)

The AIRS is mounted in a twin-turboprop aircraft (Volpar Turbo Beech 18 (VTB), range: 1800 km, cruising speed: 300 - 330 km/h), operated by ZIMEX Aviation, Zürich, Switzerland, under contract to the Biological Control Program of the International Institute of Tropical Agriculture. The AIRS was developed for the continent-wide release and delivery of the cassava mealybug parasitoid *E. lopezi*. It consists of a metal frame for mounting the release cassette with biocontrol agents, a pressure system, a tube system and an electronic operating system. A detailed description of the AIRS is provided by Bird (1986, 1987), Herren *et al.* (1987) and Drukker *et al.* (1991).

The pressure system ejects the contents of release cassette tubes into the tube system where they are decelerated to a speed less than 100 km/hr relative to the ground. The machine is operated by the pilot who pushes a button when overflying a release marker (a fixed point in or in front of the target field where the release should be activated). The electronics then trigger the pressure system with a negligible delay. An electronic eye records each ejection. The release cassette consists of 361 isolated tubes formed in a fixed (19x19) array. The tubes are ejected at a speed of about one per second or one every 83 meter at 300 km/hr. One side of the tube is permanently closed by fine metal gauze with meshes of about 176 µm. The other side is open for filling with biocontrol agents but is closed after filling by a disposable airtight stopper which is blown off by the pressure system at ejection whereupon the contents in the tube leave the plane.

Definitions

Mortality is defined as the number of dead mites. Mortality rate is the mortality as a percentage of the original amount of mites. Loss is mortality plus the number of mites not recovered. Loss rate is the loss as a percentage of the original amount of mites.

Laboratory and field trials

Most of the laboratory trials were carried out in the lab at 26°C and 55% RH, but some experiments at (33°C, 76% RH). Phytoseiid mites of the South American species *Neoseiulus idaeus* Denmark and Muma (Brasilian strain) were used for all the experiments. They could be distinguished from native phytoseiids and were available in adequate numbers from Biological Control Program's mass production unit (Friese *et al.*, 1987). Reproduction and viability parameters of a Colombian strain of this species on *Tetranychus urticae* are well documented (Van Dinh *et al.*, 1988a). The predator is relatively drought resistant in comparison to other species of Phytoseiidae (Van Dinh *et*

al. 1988b). In laboratory and field studies this mite proved to be a predator of *M. tanajoa* , but establishment in Africa is yet to be confirmed (Van den Berg & Markham, 1987, Yaninek, unpubl.).

The aerial release procedure was subdivided into steps, each of which was analyzed with respect to losses of mites. Possible premature escape of mites through the meshes in the gauze (ø: 176 µm) of the aerial release cassette's tubes was checked by storing one tube with 60 adult females and two tubes with 50 deutonymphs each overnight at 22°C. To avoid unwanted escapes in later experiments the gauze of the tubes was sealed with parafilm.

Losses during storage, not attributable to premature escape of mites, were checked by filling four tubes of the release cassette with four different carrier materials: oats, bran, polyfoam spheres (ø: 2 mm) and paper confetti (ø: 5.4 mm). These were mixed with four cultures of *Neoseiulus idaeus* (about 560 mites per culture) and eggs of *Tetranychus urticae*. Three tubes were filled with different types of carrier material (bran, 2 tubes; oats, 1 tube) and lower numbers of mites (100, 150 and 200 per tube respectively). The cassette was kept 18 hours in the polyfoam box with a cooling element before opening and counting. In this and subsequent trials counting was done by hand, removing the mites with a brush. A Berlese funnel was used to extract the remaining mites from the carrier materials.

The next step was to find out in what way mites cling to the carrier materials during release. In Africa's small and scattered cassava fields free mites can be considered as lost. The AIRS was used on the ground whereby the contents of the cassette tubes could be intercepted immediately upon ejection. The contents were led through a sieve which separated the course fraction of the carrier materials from finer particles. In this way mites that stuck to the carrier material were separated from mites that had drifted free. Sieves with pores of 2000 µm were used. As carrier materials, oats and three different types of bran (fine, middle and course flakes) were used. Ten tubes were ejected, 5 with bran of different textures mixed with 100 (4) or 200 (1) mites, 5 with oats, mixed with 75 (1), 100 (1) or 200 (3) mites.

In aerial release trials, additional properties of the carrier materials were tested. Experiments were usually prepared the day before by mixing phytoseiids with carrier material, inserting these into a tube of the release cassette and pinching the stopper on it. After filling, the cassette is stored in a coolbox at a temperature of 21-23°C until it is inserted in the AIRS. For meteorological reasons most of the experiments took place early in the morning (lower wind speeds and temperatures). During the experiments, local wind speed, wind direction and temperatures were measured regularly.

On a weekly mown strip of land (15 x 500 m), boards (60 x 90 cm) covered with fluorescent cards were placed every 15 m to indicate the flightpath to the pilot. A 3 m high triangular fluorescent release marker, 15 m left of the center line indicated the release point. Thirty to fifty meters further along the center line a fumigation sheet (15.2 x 18.3 m) was placed as a target. Later on, releases took place in a cassava field (45 by 120 m, density 0.52 plants/m^2, variety: AGRIC, planted: August 1988) supplied with the same boards and release marker. The exact drop zone of the materials relative to the fixed release marker and to the flightpath of the aircraft was mapped. On the basis of these data a minimum field size was defined.

Whether the carriers would lodge on the cassava plants was assessed by checking cassava leaves in the surroundings of grounded flakes of carrier material. The visibility and the spatial dispersion of oats (4 tubes), stained by means of several fluorescent dust colourants (Saturn Yellow and Neon Red, Swada Products, London) were compared to those of confetti (4 tubes). Dispersion patterns of confetti were compared when released on 2 m (2 tubes), 10 m (3 tubes) and 20 m (8 tubes) above the field.

Finally, a mass release of *N. idaeus* on oat flakes (26 g; 4300 flakes) was done in the cassava field using 24 cultures containing a total of 15,135 mites (4041 adult females and 11,094 others; the cultures contained 13,856 eggs) including food (*T. urticae* eggs). These phytoseiids were divided over 6 cassette tubes, preceded and followed by a tube containing confetti to mark the release area. After release, the area

was surrounded by ribbon. Samples of 50 leaves were taken every day in the first week and then at longer intervals.These were examined for the presence of phytoseiids and *Mononychellus tanajoa*.

RESULTS AND DISCUSSION

Adult mites were too big to escape through the gauze of the cassette tubes, only 3% escaped,while only 3% of the nymphs stayed in the tubes (Table 1). Clearly, when using the AIRS for releasing mites with carrier materials on a large scale, release cassettes should be furnished with finer gauze (about 80 µm).

Table 1. Escape from and mortality in the tubes of the release cassette.

tube	no. of adults	no. of nymphs	no. escaped after15 hours	mortality	alive in tube after15 hours
1	60		2	0	58
2		50	35	2	1
3		50	26	4	2

Survival of phytoseiids, stored in several types of carrier material was quite high, taken into consideration that a number of mites escaped during the process of packing the cassette. Up to 68% were recovered (Table 2). Only in tubes 3 and 4 was mortality checked, amounting to 13 (2.7%) and 45 (6.7%). For lower mite densities losses were much bigger than for high mite densities. Oats and bran seemed to be the most promising materials. Pickett *et al.* (1990), who used even lower mite densities (4 per gram grits) than we did, found a reduction in mite density of 50% after 1.5 hours, which may have been caused by other factors than mortality.

Table 2. Losses of mites after overnight storage in the cassette tubes in carrier materials.

tube	material	mass (g)	no. of particles	no. of mites at start	no. of mites recovered	% loss
1)	polyfoam	0.14	170	557	306	46
2)	oats	4.3	720	512	351	32
3)	confetti	2.3	400	487	288	41
4)	bran	2.0	3400	672	457	32
5)	bran	2.0	3400	100	16	84
6)	bran	2.0	3400	150	59	61
7)	oats	4.3	720	200	59	71

In the separation experiment only 5% (62) of 1375 mites were recovered after ejection, most of them in the fine fraction (76%). For oats this percentage was slightly more favourable than for bran (70% and 86% respectively). No differences could be established among the different types of bran. As the connection with the AIRS was not completely airtight it is probable that many more mites drifted free upon ejection. Results from the previous section indicated that the huge loss was not overnight storage mortality. Possibly the mites were incapable of holding on to the flakes when exposed to ejection pressure. This hypothesis can be checked by using carrier material with a different texture like corncob grits, which was not available at the time of the experiments.

The spatial distributions of oats/bran and confetti were quite similar. Since confetti is more visible than oats and bran, releases from higher altitudes were only done with confetti. The lodging capacities on the cassava plants of confetti and oats turned out to be rather dissappointing. Only a few oats flakes and no confetti were found lodged on the cassava plants, most had fallen on the ground.

The surface area over which the material dispersed increased with altitude while the recovery percentage decreased as a result of downwind drift and probabilistic

divergence (Table 3).

Table 3. Recovery percentage,surface area and location of aerially released confetti particles in relation to drop altitude.

drop altitude (m)	no. of particles	recovery %	surface area (m^2)	mean distance flightpath(m)	mean distance marker(m)	wind speed (km/h)
2	1100	40	340	8	21	7.6
10	1660	30	438	13	17	7.6
20	4420	17	672	17	7	10.8

The area that can be covered with one shot depends on the density of phytoseiids required for successful establishment. At this moment no data are available to illustrate this. If low densities are sufficient, phytoseiids can be released from high altitudes and large areas can be covered. It can be extrapolated that in a release from 150 m an area as large as 1600 m^2 can be covered with one particle per m^2. If high densities are required, releases have to be carried out at lower altitudes and it is probable that even at 2 m, the lowest drop altitude possible, the carrier materials will be dilluted to such an extent, that most of the phytoseiids will drop beyond many of Africa's small cassava fields, which rarely exceed 340 m^2. In that case containers which confine the predators are preferable (Drukker *et al.*, manuscript).

The surface area covered by oats when used as a carrier for the mites in the mass release trial was 340 m^2. Only 2 of the 15,000 mites released aerilly were recovered in the cassava field (Table 4). The difference between our results and those of Picket *et al.* (1987), who used similar amounts of phytoseiids (*Phytoseiulus persimilis*) per area can be explained in various ways. 1. The lower speed of their aircraft may have caused lower mortality due to lower gravitation forces. 2. The carrier materials they used may be more suitable. 3. Their experimental fields were larger, which reduced losses due to downwind drift and enabled them to release on a larger scale. 4. Their phytoseiids were able to establish and to found growing populations in the corn field ecosystem which made sampling easier.

It can be concluded that the reliability of this method can not be fully evaluated before a phytoseiid is found that is capable of becoming established in cassava fields. This method is suitable for dispersing mites in low densities over very large areas, but not to target high concentrations of mites on one single spot.

Table 4. Number of phytoseiids recovered after aerial release of 15,000 mites with carrier material in a cassava field with *M. tanajoa*.

days from start exp	no. of phytoseiids found	no. of *N. idaeus* recovered	no. of *M. tanajoa* per leaf
1	5	0	17
2	2	0	20
3	14	0	22
4	1	0	6
6	10	1	23
8	23	0	24
14	2	0	24
16	25	0	30
24	25	1	18
37	48	0	15
42	29	0	13
97	2	0	0
124	0	0	0
153	0	0	0

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DIAPAUSE INDUCTION IN THE THRIPS PREDATORS *AMBLYSEIUS BARKERI* AND *AMBLYSEIUS CUCUMERIS* (ACARI: PHYTOSEIIDAE) IN DUTCH GREENHOUSES

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Keywords:
Amblyseius cucumeris, Amblyseius barkeri, biological control, diapause.

Summary

The predatory mite *Amblyseius cucumeris* (Oudemans) is used for biological control of thrips on greenhouse-grown sweet peppers. Preliminary greenhouse experiments showed that females of *A. cucumeris* entered a reproductive diapause when exposed throughout juvenile development to greenhouse conditions from 13 February until 6 March. When mites were exposed to an artificial long-day photoperiod in the same greenhouse no diapause occurred.

A strain of *Amblyseius barkeri* (Hughes) (called 'P'), collected from a sweet pepper crop during winter, demonstrated a high percentage non-diapausing females under both short-day and artificial long-day conditions under greenhouse conditions. Laboratory experiments showed that complete diapause was induced in the commercially available strains of both *A. barkeri* and *A. cucumeris* under short-day conditions (LD 10:14 photoperiod and TC 23°:16° thermoperiod) while the diapause incidence was found to be only 51% in the *A. barkeri* 'P' strain.

In contrast to what has been found for *A. cucumeris*, diapause can be induced in *A. barkeri* under short-day conditions on a diet of pollen of *Vicia faba* without a supplement of ß-carotene. Because of its diapause characteristics the *A. barkeri* 'P' strain might be a good agent for biological thrips control on sweet pepper crop during winter.

INTRODUCTION

The predatory mites *Amblyseius cucumeris* (Oudemans) and *Amblyseius barkeri* (Hughes) are produced commercially as agents for biological control of two species of thrips, *Thrips tabaci* Lindeman and *Frankliniella occidentalis* (Pergande), in commercial

greenhouses. Ramakers (1988) showed that on sweet pepper, *A. cucumeris* established itself easier and reached higher population densities than *A. barkeri*. He considered *A. cucumeris* a better predator for thrips control on this crop. Today *A. cucumeris* is effectively used in controlling thrips on sweet pepper crops during spring and summer months. The success of biological control on sweet pepper can be explained by the presence of pollen as an alternative food source for predatory mites (Van Rijn & Sabelis, 1990). This pollen even makes a preventive introduction possible (Ramakers, 1990).

Morewood & Gilkeson (1990) demonstrated that *A. cucumeris* enters diapause in Canadian greenhouses in autumn when daylength becomes shorter than 12.75 h and greenhouse night temperatures are below 21°C. In Dutch greenhouses, *A. cucumeris* failed to become established on sweet peppers in February and March. *A. barkeri*, however, sometimes occurred spontaneously on sweet pepper during this period (K. Altena, pers. comm., 1990). It is not known if this species also shows a reproductive diapause during winter period.

The present paper describes the influence of greenhouse conditions during winter on diapause induction in the commercially available strains of *A. cucumeris* and *A. barkeri* and in a strain of *A. barkeri* collected from a sweet pepper crop during winter.

MATERIAL AND METHODS

The strain of *A. cucumeris* originated from the stock rearing of Koppert B.V.(Berkel en Rodenrijs, The Netherlands), which is used for the inoculation of mass rearing units. One *A. barkeri* strain is a sample of the laboratory strain, reared in the Glasshouse Crops Research Station at Naaldwijk, The Netherlands, obtained in January 1989. The commercial strain of *A. barkeri*, used by Koppert B.V., was derived from this laboratory strain. The other strain of *A. barkeri* (called 'P') originated from sweet peppers in a greenhouse where no *A. barkeri* had been introduced.

All strains were reared on pollen of the broad bean, *Vicia faba* L., for about half a year before testing. This pollen diet is equivalent to live prey, like thrips larvae, as regards development and reproduction of the predacious mites (Van Rijn & Van Houten, 1991). For the experiments on diapause, the diet of *V. faba* pollen needs to be supplemented with ß-carotene, since *A. cucumeris* fed on broad bean pollen alone ceases to respond to photoperiod (Overmeer *et al.*, 1989). Complete restoration of the ability to diapause is obtained when the diet is enriched with 5 mg of ß-carotene (Merck, synthetic, crystalline) per 100 mg of pollen. The mites used in the greenhouse experiments were reared on this ß-carotene supplemented pollen diet.

The experiments were carried out on rectangular "arenas" made of black plastic. Wet tissue paper was wrapped over the edges of the arena, serving both as a barrier and a water source. An additional barrier of tanglefoot on the tissue paper prevented the mites from escaping. The experiments were started by placing eggs from 0 to 24 h old on an arena provided with a small roof-shaped shelter made of thin transparent plastic foil, under which the mites, once hatched from the eggs, could hide.

For the duration of the experiments in the greenhouse, the arenas were placed on the ground between the sweet pepper plants in one part of the greenhouse. An artificial long-day photoperiod was created by giving supplementary light from 1.30 a.m. until afternoon. Radiant flux density at the level of the mites on the arenas was not measured. The thermoperiodic regime in the greenhouse during the experiments was as follows: the temperature decreased from 22°C to 16.5°C between 4 p.m. and 6.30 p.m. and was maintained on 16.5°C until 1.a.m.. Between 1 a.m. and 3 a.m. temperature increased to 19°C and was maintained at 19°C until 6.30 a.m.. Between 6.30 a.m. and 8 a.m. the temperature increased to 21°C. During daytime, the temperature fluctuated between 22°C and 25°C, depending on the outside temperature and the sunshine.

The most conspicuous characteristic of diapausing females is that they do not produce eggs. In addition to this, diapausing females try to find an overwintering place. As a result, most of these females run into the tanglefoot barrier whereas nearly all non-diapausing females stay on the arena. Absence of egg production before running into the glue was therefore taken as the criterion of diapause. In each experiment it was ascertained that enough males were present to inseminate all females.

In the laboratory experiments the arenas were placed in photoperiod and thermoperiod controlled incubators. Temperatures were maintained within ± 0.5°C. Temperature transitions in the thermoperiodic regimes took about 1 h. From each strain two groups of mites were tested. One group was reared on a diet supplemented with ß-carotene and one control group on a diet lacking the supplement of ß-carotene. Due to the lack of carotenoids, it was expected that the control groups would not respond to photoperiod or thermoperiod (Van Houten et al., 1987; Overmeer et al., 1989). The control mites served to determine the start of the oviposition period.

Two days after the first adult females appeared, the mites were fed with pollen of the iceplant, *Dorotheanthus bellidiformis* N.E. Brown, which has also been shown to be an adequate food source for *A. cucumeris* (Overmeer et al., 1989) and *A. barkeri* (unpublished results). In this way egg production by individual females could easily be determined, as the intestines of mites feeding on iceplant pollen become purple-coloured. In non-diapausing females the white egg stands out clearly against the surrounding purple coloured intestines; diapausing females, on the other hand, do not feed and remain pale.

RESULTS

Diapause under greenhouse conditions during winter

The results in table 1 show that complete diapause was induced in *A. cucumeris* when the mites were reared under short days in the greenhouse. Most of the females ran into the glue during the last week of the experiment, without laying eggs. Under the same conditions, diapause was virtually absent in *A. barkeri* 'P'. Besides eggs, larvae and nymphs, a lot of females were present on the arena at the end of the experimental period. No diapause was observed in the control experiments carried out under the artificial long-day photoperiod in both species of predatory mite.

Date mites	short-day photoperiod		artificial long-day photoperiod	
	A. cucumeris	*A. barkeri* 'P'	*A. cucumeris*	*A. barkeri* 'P'
13/2/1990				
egg	(200)	(118)	(110)	(118)
28/2/1990				
female	(> 25)	(> 25)	(> 15)	(> 25)
male	+	+	+	+
egg	-	+	+	+
6/3/1990				
female	(2)	(23)	(13)	(23)
male	+	+	+	+
egg	-	+	+	+
larva	-	+	+	+
nymph	-	+	+	+

Table 1. Effect of photoperiod on diapause incidence in *Amblyseius cucumeris* and *Amblyseius barkeri* 'P' reared under greenhouse conditions during winter. (N): number of females or eggs present. +: present, -: absent.

Diapause under short days on different diets

To investigate the diapause incidence in *A. cucumeris* and both strains of *A. barkeri* under short-day conditions, mites were reared in the laboratory on a diet of *Vicia* pollen with and without a supplement of ß-carotene. Full diapause was induced in *A. cucumeris* and *A. barkeri*, whereas only 51% diapause was induced in *A. barkeri* 'P' when the mites were fed on *Vicia* pollen supplemented with ß-carotene (Table 2).

Remarkable was, however, that diapause occurred in both *A. barkeri* strains on a diet of *Vicia* pollen without the supplement of ß-carotene.

Species/Strain	Diet	% Diapause	Number of females
A. cucumeris	*V. faba*	0	30
	V. faba + ß-carotene	100	38
A. barkeri	*V. faba*	100	81
	V. faba + ß-carotene	100	33
A. barkeri 'P'	*V. faba*	47	57
	V. faba + ß-carotene	51	110

Table 2. Incidence of diapause in a strain of *Amblyseius cucumeris* and two strains of *Amblyseius barkeri* under short-day conditions (LD 10: 14 photoperiod and TC 23°: 16° thermoperiod) reared on different diets. (L = photophase, D = scotophase, T = thermophase, C = cryophase).

DISCUSSION

Both commercial species of predaceous mite, *A. cucumeris* and *A. barkeri*, used in Dutch greenhouses for biological control of thrips, appear to enter diapause when they are introduced during early winter, despite the gradual increase in daylength which takes place in this time of the year (Table 1). *A. barkeri* 'P', however, shows a high incidence of non-diapause when reared under these conditions. Although the commercial strain of *A. cucumeris* is thought to be a better thrips predator on sweet pepper than *A. barkeri* (Ramakers, 1988), this is not a consequence of the presence or absence of pollen, since it has recently been shown that both species can survive on pollen alone (Van Rijn & Van Houten, 1991). Besides, native *A. barkeri* is quite common on sweet pepper, especially in late season, whereas *A. cucumeris* seldom occurs spontaneously (Ramakers, 1988). When growers want to use phytoseiid mites during the winter, the *A. barkeri* 'P' strain might be the best candidate for this period because of its lowered incidence of diapause. Another solution might be that growers create an artificial long-day photoperiod during winter to prevent diapause incidence in *A. cucumeris* (Table 1), as long as non-diapausing strains of *A. cucumeris* are not available. For the predacious mite *A. potentillae*, it has been demonstrated that the light sensitivity threshold for the photoperiodic induction of diapause is very low (Van Houten & Veerman, 1990).

Experiments to investigate diapause prevention in *A. cucumeris* and *A. barkeri* by low-intensity light are in progress.

The fact that *A. barkeri* responds to a combined photo/thermoperiodic regime on a diet of *Vicia* pollen alone does not mean that ß-carotene may not be involved in the photoperiodic and/or thermoperiodic response in this species. Some alternative explanations have been given for another predator, *Typhlodromus pyri* Scheuten, which also demonstrated a complete diapause on a diet of *Vicia* pollen alone under a short-day regime (Overmeer et al., 1989). Whether *A. cucumeris* and *A. barkeri* also respond to a photoperiodic and/or thermoperiodic regime on a diet of sweet pepper pollen has still to be investigated.

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NATURAL ENEMIES OF *JACOBIASCA LYBICA* (DE BERG): A LITERATURE SURVEY

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Keywords:
Jacobiasca lybica, natural enemies, biology, literature search.

Summary

A literature search was done to collect information and review the biology and the natural enemies of *Jacobiasca lybica*, a serious cotton pest in Sudan. Based on this review, the possibilities for biological control of this pest can be estimated. Many articles deal with the biology of *J. lybica*, but the information is old and often contradictory. Very little is known about natural enemies occurring in Sudan and surveys are therefore needed. Natural enemies worth investigating for their possibility as a biological control agent of *J. lybica* are: *Anagrus atomus* and *Erynia radicans*.

Introduction

Jacobiasca lybica is a small and plant-juice sucking insect belonging to the Cicadellidae (Homoptera). It is polyphagous, and causes a lot of damage. It punctures the lower epidermis and mesophyl cells are stung, torn, disordered and filled with salina sheeth material by the stylets on their way to the phloem. The leaf turns yellow and the edges start curling. This phenemona is called 'hopperburn' (23).

Jacobiasca lybica is a pest on many important crops such as cotton, eggplant and grape vine. It is found in the major part of northern Africa (north from Tanzania and Uganda), in parts of Asia and southern areas of Europe. It is controlled chemically and since the 1950 ties, the number of applications have increased dramatically. E.g. in Sudan the spray frequency increased from one per year in the fifties to eight or nine in 1980 (7, 25). An alternitive for the chemical control of *J. lybica* might be biological control. To investigate the possibilities of biological control, an excessive literature surch is needed to obtain information about the biologyof the pest and its natural enemies. This paper summerizes the results of such a literature search.

The search was directed towards *J. lybica* occuring in Sudan. The name however has been revised recently. It used to be *Empoasca lybica*, and a lot of authors still use this name. Some authors refer to it as *Austroasca lybica*. In this review the name used by the CAB will be considered the correct name, which is *J. lybica*. Other economically important species within the genus *Empoasca* were revised recently also, now belonging to the genus *Amrasca* (*A. biguttulla biguttulla* or *A. kerri* for example). Natural enemies of these genera might be natural enemies of *J. lybica*. That is why natural enemies of species belonging to the genera *Jacobiasca*, *Austroasca*, *Empoasca* and *Amrasca* are included in this review.

Material and methods

The literature search was mainly done by computer. Bibliographies of references obtained by this search were used to search for other and older references. Most computer data bases do not contain references before 1973.

Some of the inexpensive databases were first checked: Phytomed, the on-line version of Bibliografie der Plantzenschutz Literatur, and Agris, the on-line version of the the FAO's Agrindex. Keywords used were: the genus and species name, common names

(green leafhopper, jassid and cotton jassid), cotton, the genus name of cotton, *Gossypium* and combinations of these. It resulted in 128 references. These titles and, when possible, abstracts, were checked and articles of apparent interest were selected and obtained through the library. Information obtained from these articles and those collected through their bibliographies resulted in enough information to create a much more detailed literature search. Most synonyms of *J. lybica*, closely related pest species and many natural enemies were now known. This information, combined with more general keywords (like natural enemies) was used to create a detailed literature search to explore the larger and more expensive on- line databases of CIBC, Biological Abstracts and Biological Abstracts/ RRM. Later on Agricola and Current Contens on CD-rom were checked. The most detailed search was done using the CIBC database, which revealed most new entries: 188 entries. Biological abstracts revealed just 65 entries. Both databases on CD-rom did not reveal any new references. References of interest were collected through the library, and together with articles obtained by backtracking the bibliographies and manual search in recent publications, resulted in an literature review containing 127 articles of interest to the subject.

Results and discussion

Biology

Jacobiasca lybica is elongated, wedge-shaped and approximately 2.5 mm in length. The body is pale green with semi-transparent, shimmering wings (22). It is polyphagous. In Sudan more than 60 plants have been recorded as host plant of this pest (4, 5 16, 22). The eggs are laid preferably near the insertion point of the leaf petiole or roughly halfway along the midvein, embedded in the cortex. Young and old leaves are avoided (8, 16, 18). The eggs are greenish, relatively large and are cylindrical in shape. They are slightly curved and are broader and bluntly rounded at the posterior end and somewhat taper anteriorly (4, 10, 22). The emerging nymph is initially colourless and later becomes yellowish-green (4). It is frog-shaped and flattened. It is characterized by a rapid crablike sideways movement when disturbed. It remains at the underside of the leaf (22). It normally undergoes 5 instars before reaching maturity, but at temperatures between 27°C and 29°C, 6 instars are possible (8, 10, 18).

In Sudan populations of *J. lybica* build up as soon as the first cotton seedlings appear. Build up of populations are fast, especially during the rainy season and subsequent hot weather. Peak populations occur at the end of November and December (3, 4, 16). At the end of February only a few individuals remain. Six generations have been recorded in Sudan.

More biological data of *J. lybica* are summerized in table 1. Publications provide conflicting data, so a lot of additional research is needed to obtain a clear idea of the biology of this pest.

Table 1: Biological data of *Jacobiasca lybica*.

adult longevity	2 weeks - 2 months	4, 10, 16, 18
sex ratio (male:female)	1:1 - 1:2	10, 16, 18
preoviposition period	1-4 days (summer)	10, 16, 18
	up to 3 weeks (winter)	18
eggproduction per day	1-7	18
fecundity	60-80 eggs	10, 16, 18, 22
developmental time		
egg stage	5-15 days	4, 10, 16, 18, 22
nymphal stage	8-12 days	4, 10, 16

Jacobiasca lybica causes hopperburn. At the Gezira agricultural scheme in Sudan, spraying is started at population levels of 100 ind./100 leaves (12). Stam et al. (1988), concluded that infestations of 140 ind./100 leaves in November, and 200 ind./100 leaves in December were tolerated by the cotton plant. *J. lybica* in Sudan is of economic importance between 1 September and 15 December (16). Initial infestation of cotton is caused by short-distance migration from fallow land and long-distance migration may also be important.

Control

Control of *J. lybica* is mostly achieved by using pesticides. The number of applications has increased dramatically over four decades, and so did the costs. As a side effect, natural enemy populations are reduced significantly by these treatments (25). Herrera (1986) compared the predators recorded during a cotton field survey, with those recorded before synthetic organic pesticides were used (1925-1945). A dramatic decline in the number of predator species was discovered, causing a biotic vacuum. Control by means of resistant varieties of host plants have been tried with varying succes. Hairy cotton for example, is known to cause resistance, but does not seem to be effective at the Gezira in Sudan. Besides, another major pest, the whitefly *Bemisia tabaci*, prefers to oviposit on hairy cotton, so hairy varieties are more susceptible to whitefly attack.

Biological control may be another possibility to control *J. lybica*, and its possibilities are now investigated. There are hardly any records of natural enemies of *J. lybica* besides the ones from Sudan, and even these are not complete. Predators recorded from Sudan are: *Coccinella rufescens* (Muls.), *Exochamus nigromaculatus* (Muls.), which are both Coleoptera: Coccinellidae, *Chrysopa vulgaris* (Neuroptera: Chrysopidae), and some unidentified spiders (11,13). Joyce (1961) recorded coccinellids, chrysopids, *Orius* sp. and a species of predatory thrips, but there is clearly a lack of information. Parasites found to be connected with *J. lybica* in Sudan are: a pipunculid fly (Diptera), *Anagrus* sp. (Hym.: Mymaridae) and *Aphelopus* sp. (Hym.: Dryinidae) which is most abundant (13, 22).

Potential natural enemies

Other potential natural enemies were looked for. All natural enemies of *Jacobiasca* spp., *Austeroasca* spp., *Empoasca* spp. and *Amrasca* spp. found in literature, were recorded and screened for their possibilities as a biological control agent, by comparing them with the criteria from table 2, obtained from van Lenteren (1986).

Table 2: Criteria for pre-introductory evaluation of natural enemies.

	release programme		
	inoculative	*seasonal inoculative*	*induntative*
Seasonal synchronization with host	+	-	-
Internal synchronization with host	+	+	-
Climatic adaptation	+	+	+
No negative effects	+	+	+
Good culture method	-	+	+
Host specificity	+	-	-
High reproductive potential	+	+	-
Good density responsiveness	+	+	±

The parasites recorded can be devided in two groups, those attacking the egg (Mymaridae and Trichogrammatidae, which are both Hymenoptera) and those attacking

the nymphal or adult stages (Dryinidae; Hymenoptera, and Pipunculidae; Diptera). Egg parasitism has the advantage that the pest species is killed before it can exert any damage, but very high percentages egg mortality have to be obtained. Mymarids seem to be very good potential biological control agents, and several introductions in new areas are known (9, 15, 28). Most mymarids parasitizing *Empoasca* spp. belong to the genus *Anagrus*. At least eight different species of *Anagrus* and nine other species of Mymaridae, have been recorded to be parasitic on *Empoasca, Jacobiasca*. and *Amrasca*. Of these species *Anagrus atomus* seems to be the best candidate for introduction into Sudan, because it has already been recorded on *J. lybica*, and very high parasitism levels are recorded from Italy (1, 26, 27). However, its extensive host range (which includes several orders), should be tested first to eliminate the change of attacking benificial insects.

Only a very limited number of species of the Trichogrammatidae are reported to be parasitic on the target genera. Only three species, belonging to different genera, were reported to be parasitic on *Empoasca* or *Amrasca* spp. The information is too limitted, so further investigations are necessary to test their suitability as a biological control agent of *J. lybica*.

All species of Dryinidae recorded to be parasitic on *Empoasca, Jacobiasca* or *Amrasca* belong to the genus *Aphelopus*. At least eight different species were recorded. They deposit the eggs internally. During the second instar, the larva pushes out and forms a larval sac. No host defense has been observed and parasitism levels can be quite high. Much more information is still needed such as temperature adaptation and diapauze. The best candidate to intoduce in Sudan seems to be *A. witei* because it is already recorded to attack *J. lybica*.

Pipunculidae are the only Diptera to be associated with plant- and leafhoppers. Of the Pipunculidae at least eight (sub)species are recorded to attack *Empoasca* spp., all belonging to the genus *Chalarus*.Eggs are laid internally and there are two larval instars. They are extremely difficult to rear or keep in the laboratory. Parasitism levels can be quite high. All recorded species diapauze and are recorded from Europe. It is unclear whether they are adapted to the temperature regimes in Sudan, or whether development is synchronized with *J. lybica*. They are highly species specific, so it may be hard to discover a species which attacks *J. lybica*. *Chalarus* does not seem a good biological control agent.

In addition, it should be mentioned that most groups of parasites mentioned above are very hard to identify. Among the genera and species mentioned in the literature, several are probably misidentified.

Five entomopathogenic fungi are reported of *Empoasca* and *Jacobisca* spp. of which four belong to the Entomophthoraceae and one to the Hyphomycetes. The Entomophthora which seems to be the most important is *Erynia radicans* (Brefeld) Humber. Epizootics from this species are recorded to reduce populations of *Empoasca fabae* (Harris) dramatically in the USA and annual epizootics are recorded in Israel (2, 20). It has a wide host range, which includes species from seven orders, but it may be possible that individual strains have a much more limited hostrange. It has been found on *J. lybica* also (17). Furthermore it can be mass cultured very easily on artificial media, and houseflies can be used as a vector. Nowadays commercial techniques are available for the development of *E. radicans* as a mycopesticide (24). The main problem however may be the humidity, because high relative humidity is needed for sporulation. This problem can be solved if *E. radicans* is released during irrigation. Furthermore the correct strain should be isolated to prevent negative side effects, caused by a wide host range. *E. radicans* is by far the most promising entomopathogenic fungus, and it should be taken into serious consideration to use this fungus as a biological control agent in Sudan.

Many predators are recorded to feed on *Empoasca, Jacobiasca* or *Amrasca* spp.: 39 species of Insecta, including 6 orders and 14 famillies and 14 species of Arachnida. Most predators are general feeders, but the hymenopteran *Crabro davidsoni* Sandh was found to feed mainly on leafhoppers, restricted to three genera (6). *Crossopalpus* sp. (Diptera: Empididae) was found to feed voraciously on *Amrasca kerri* (Pruthi) and other plant- and leafhoppers in India (21). It is hard to quantify the impact of the general feeders, when put in a new environment. It seems that most general predators, which were tested for

their preferene, would prefer aphids to leafhoppers. Aphids are slow, do not flee and are easy to catch, while leafhoppers move rapidly when disturbed. Therefore, chrysopids, nabids and coccinellids do not seem to be very suitable for introduction in Sudan. Besides, many related species already occur in Sudan. It seems preferable to change agricultural practices so that general predators can re-infest cotton fields and excert stronger control. Introduction of predators whith a more limited host range are of more interest, such as *Crabro davidsoni* and *Crossopalpus*.

Conclusion

Overall it can be concluded that the available information concerning the biology is conflicting and vague and more studies are necessary. The natural enemies in Sudan itself are not well known, so before introduction of any natural enemy is considered, the local natural enemies should be known as well as their role in reducing *J. lybica*. Natural enemies which seem to be most promishing are species of *Anagrus* (especially *A. atomus*) and the entomopathogenic fungus *Erynia radicans*.

More detailed information can be found in Klerks & Van Lenteren (1990), an FAO funded *J. lybica* review, which covers all the information of the literature search, including complete lists of natural enemies and a complete annotated bibliography.

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KEYWORDS