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HOST SELECTION, HOST DISCRIMINATION AND FUNCTIONAL
RESPONSE OF APHIDIUS MATRICARIAE HALIDAY
(HYMENOPTERA : BRACONIDAE), A PARASITE OF THE
GREEN PEACH APHID, MYZUS PERSICAE (SULZ.)

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Summary

Aphidius matricariae is an important parasite of *Myzus persicae*. *Myzus persicae* frequently reaches pest status in paprika (*Capsicum annuum* L.) crops, therefore we consider to use *Aphidius* against aphid pests.

In order to obtain information about the control capacities of this parasite and to develop mass-rearing and introduction methods, the biology of *Aphidius* was studied. The parasite does not select larvae of a specific host stage for oviposition, but accepts all larvae. The wasp does distinguish parasitized from unparasitized hosts and shows an increasing rate of parasitization at increasing host density.

Introduction

In paprika (*Capsicum annuum* L.) crops relatively many pests species occur compared with tomato (*Lycopersicon esculentum* L.) and cucumber (*Cucumis sativus* L.) crops (Van Lenteren et al., 1977). Therefore it is difficult to develop a biological control programme for this crop. For only one of the six most important arthropod pests a satisfactory biological control method is available, i.e. *Phytoseiulus persimilis* A.-H., against the two spotted spider mite (*Tetranychus urticae* Koch). At this moment natural enemies of *Thrips tabaci* Lind. and *Myzus persicae* (Sulz.) are studied.

Myzus persicae is the most important aphid pest in paprika crops in Holland. The aphid is usually abundant at the moment that most fruit is harvested. Due to frequent spraying, *Myzus* became resistant against several insecticides. At present *Myzus* is chemically controlled by pirimicarb, sometimes biologically by the parasite *Aphidius matricariae* Haliday.

Aphidius matricariae seems to be a suitable parasite for the following reasons:

1. the parasite can be used during the whole growing season, it shows no diapause,
2. the wasp has a short generation cycle,
3. oviposition frequency is high, and
4. searching efficiency is high, even at low densities.

The aphid *Myzus persicae* shows in glasshouses only pathogenetic multiplication. Both winged and wingless females occur, the individuals with wings developing predominantly at high population densities. The aphid females are viviparous, i.e. larvae are produced. Four larval stages can be distinguished. The developmental time from larviposition to offspring producing females is 9 days at about 21°C.

The solitary endoparasite *Aphidius matricariae* deposits eggs into the body of the aphid with her ovipositor. From this egg a larva emerges. Five larval stages occur (Vevai, 1942). Adult aphids can still produce eggs for some days after being parasitized. The host dies nine days after parasitization and the parasite then pupates within the cuticle of the host. Total development from oviposition to adult wasp takes 3 days at 21°C. *Aphidius* is momentarily used by some growers in Holland, although few biological data about the parasite are available. A satisfactory mass-rearing method has not yet been developed, and further the proper moment and method of introducing the wasp in the glasshouse are not determined. Therefore the following aspects of this parasite-host relationship were studied.

1. Does *Aphidius* prefer larvae of one or more of the developmental stages from *Myzus persicae* for oviposition (host selection)?
2. Is *Aphidius* able to distinguish parasitized from unparasitized hosts (host discrimination)?
3. Does *Aphidius* show an increasing rate of parasitization as host density increases (functional response)?

Material and methods

Paprika plants were reared on 'water tables' (Verschoor-Van der Poel & Van Lenteren, 1978). To prevent infestation, clean plants were put into insect cages. Some of the plants were used for an aphid culture. The parasites were reared in another room. Usually we isolated parasitized hosts in vials before the wasps emerged. All rearings were done at 21°C, all experiments at 20°C.

The *Aphidius* females we used for the experiments were 24 hours old at most. Before being used, the females were put together with males until

mating was observed, and then transferred to a petri dish with some hosts. After they had parasitized several hosts, the larvae were considered to be experienced. This 'training' period is necessary for obtaining reliable data from the relevant experiment (Samson-Boshuizen et al., 1974; Van Lenteren, 1976).

To determine the degree of parasitization, hosts were dissected after the parasite larvae emerged. Eggs are extremely small and thus difficult to find. By dissecting hosts that contain larvae we might, however, have missed some eggs that did not develop.

Specific technical procedures are described later.

Results and discussion

Host selection. Larval stages of *Myzus persicae* are difficult to distinguish. We therefore divided the larvae arbitrarily in three groups: small larvae (smaller than 0.8 mm in length), intermediate larvae (0.8 to 1.2 mm) and large larvae (larger than 1.2 mm). For the experiment 5 larvae of one group were offered together with 5 larvae of another group to the parasite. The aphids were put on a sandwich of parafilm^R, between which an artificial diet was present (for details, see Harrewijn, 1977). The parafilm^R sandwich was attached to a perspex ring with an inside diameter of 4 cm, so the surface on which the aphids were present was 12.56 cm².

Thirty observations were made, each combination of sizes (small - intermediate (1), small - large (2), intermediate - large (3)) ten times (Table 2). The observations lasted 20 minutes and the behaviour of the parasite and hosts was continuously observed. For each observation a new female was used.

Aphidius searches by drumming with her antennae on the leaf surface while walking. It is not yet known whether she detects the host by seeing, smelling, tasting or feeling it. After all observations we have the impression that the parasite walks at random over the leaf and discovers a host mostly by touching it with her antennae, and less frequently by seeing the host. After contact with the antennae the wasp may sting the host with her ovipositor and lay an egg. Oviposition takes less than a second, which makes it difficult to determine what actually happens. Most eggs are deposited in the aphid's thorax plus abdomen, although oviposition also occurs in the head or in the legs. The percentage of stings resulting in oviposition is very similar for all oviposition sites (Table 1).

Table 1. Number of stings with the ovipositor by *Aphidius matricariae* in head, legs or abdomen of *Myzus persicae* and the resulting percentage parasitization. Each host was stung only once.

	head	legs	thorax + abdomen
number of stings	43	105	255
% resulting in parasitization	25	27,5	20,1

Table 2 presents the results of the host selection experiment. If *Aphidius* meets a certain host for the first time, she will sting it with her ovipositor at 95% of the cases, irrespective of host size. Large and intermediate sized larvae are met more frequently than small larvae, because of their larger size.

The number of stings per contact is larger in large larvae than that in smaller larvae. This causes the longer time spent with larger larvae. The higher number of stings in large larvae may be caused by problems in penetrating the thicker cuticle of old hosts, so more attacks per successful oviposition are necessary.

Aphidius meets and stings larvae of the three size classes frequently: apparently there is no host selection, neither from a distance (by sight or smell, resulting in a not proportional number of contacts with a certain size class) nor at contact (by rejecting larvae of certain size classes more frequently than others after contact with antennae or ovipositor).

In larvae of all size classes a rather high percentage of parasite survival was measured, although ovipositions in large larvae resulted less frequently in an adult parasite (Table 2).

Conclusion: although no clear preference by *Aphidius* for larvae of certain size classes of *Myzus* was measured, it might be preferable for *Aphidius* to parasitize small larvae because

1. it takes less time to parasitize small than large larvae,
2. the percentage eggs resulting in adult parasites is higher if oviposition takes place in small larvae than when it takes place in large ones, and
3. small parasitized larvae do not produce aphid larvae, whereas older larvae may start to larviposit after moulting to the adult stage before they die.

Table 2. Number of contacts with, stings in, average time spent with, and percentage parasitization of larvae of three sizes of *Myzus persicae* by the parasite *Aphidius matricariae*.

average no. of encounters per 20 min.	comb. 1	21.6	25	
	2		18.5	25.3
	3	13.3		23.7
average no. of stings per 20 min.	comb. 1	12.5	20.1	
	2		21.1	33.8
	3	13.7		27.2
average no. of encounters that resulted in at least 1 sting, per 20 min.	comb. 1	8.9	12.1	
	2		8.9	12.9
	3	7.5		10.8
percentage of the observation time spent with hosts of a certain kind	comb. 1	36.8	63.2	
	2		30.3	69.7
	3	30.9		69.1
percentage parasites that emerged from parasitized hosts	comb. 1	100	94.9	
	2		89.5	77.8
	3	81.2		66.7

Host discrimination.

Two different experiments were done to study whether *Aphidius* can distinguish parasitized from unparasitized hosts (for definitions and analysis of host discrimination, see Van Lenteren et al., 1978).

First experiment:

In the first experiment we used the same perspex ring and parafilm^R surface as described at the host selection experiment. On the parafilm 5 unparasitized and 5 parasitized (once stung) hosts were put. To distinguish between the two kinds of host, one group was marked with a small dot of Indian ink. This mark did not influence the wasp's parasitization behaviour (see Fig. 1). The wasp and the hosts were

observed continuously and the experiment was terminated after about 25 stings. Every host that was parasitized during the observation was removed and replaced by a fresh one of a similar kind. Fifteen replicates were done, with 15 different females. For the collection of parasitized hosts needed for the experiment other females were used.

Figure 1 gives the results. Although the number of contacts with parasitized and unparasitized hosts is the same, the number of stings with the ovipositor differs. Unparasitized hosts are stung twice as often as parasitized ones and from this we may already conclude that the parasite distinguishes between unparasitized and parasitized hosts.

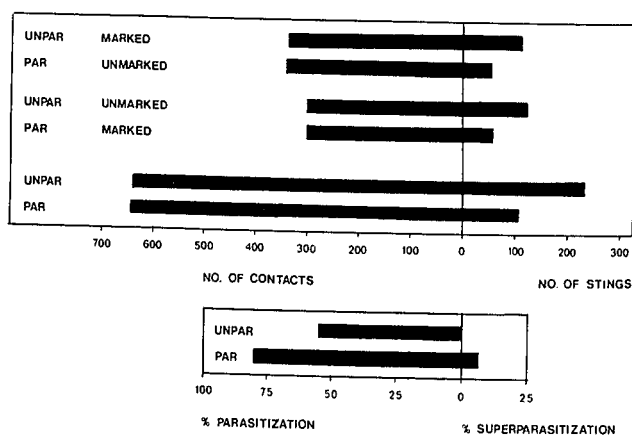


Fig. 1. Number of contacts with and parasitizations in unparasitized and parasitized larvae of *Myzus persicae* by *Aphidius matricariae*, and percentage parasitization and superparasitization (In the upper part of the first graph the result for the two replicates is given).

Although the wasps discriminate, still 16.3% of the already once stung hosts ('parasitized') was stung for a second time. One of the causes of this may be that not all hosts that were stung once were really parasitized, and that a part of the hosts did not contain an egg. Stinging with the ovipositor takes less than one second, so the observer can not determine whether oviposition occurs. The data about stings in unparasitized hosts show that after one sting only 55% is actually parasitized. We may therefore suppose that only 55% of the once 'parasitized' hosts contained an egg. So if at the second contact a sting was observed, it does not necessarily mean that a superparasitization took place, but that a first oviposition occurred. In deed we see (Fig. 1) that 80% of the twice

stung hosts contain one egg, and only 6 percent contains 2 eggs.

Vevai (1942) described a similar phenomenon; he found that:

after 1 sting	23.2%	of the larvae	contains	an egg
" 2 stings	45.6%	"	"	"
" 3 stings	80 %	"	"	"
" 4 stings	100%	"	"	"

Second experiment

In the second experiment 10 unparasitized hosts were put on the parafilm^R surface. A wasp was introduced and observed continuously for 20 minutes. Thirty tests were done; for each test another female was used.

In Fig. 2 the percentage contacts resulting in acceptance, i.e. antennal contact followed by stinging, and percentage contacts leading to rejection, i.e. antennal contacts not followed by stinging, is given. Although we used the terms acceptance and rejection, it should be realized that not every sting (acceptance) results in egg laying, so some acceptances might be rejections. The main aim of this test, i.e. showing the ability of host discrimination of the parasite *Aphidius* allows this simplification.

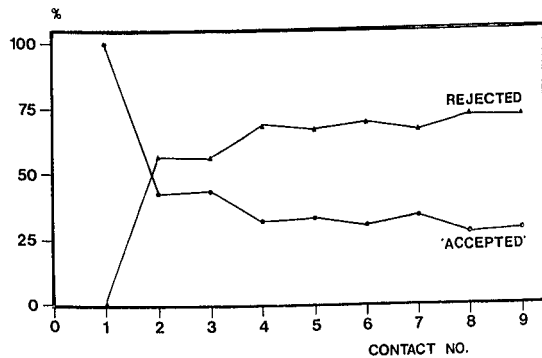


Fig. 2. Percentage of hosts accepted (●) or rejected (▲) against contact no.

Sometimes rejection of a host occurred at the very first encounter; in those cases we considered the first encounter that led to stinging as first contact. The figure shows that the percentage acceptance decreases with an increase in the number of encounters, the percentage acceptance being 43.0 percent at the second visit and 28.2 percent at the ninth. The percentage acceptance does not decrease to 0. This is caused firstly by the already mentioned fact that not all stings result in oviposition,

and secondly by the fact that after the parasite tested the host once or twice with the antennae only, a sting may follow at a succeeding encounter. That the wasp meets the host so frequently is due to the artificial conditions of this experiment. On top of the perspex ring a piece of glass was placed and this forced the wasp to stay with the hosts for 20 minutes, while most hosts were already stung after 200 seconds. We saw, however, that the wasp frequently attempted to escape (see for the same phenomenon: Van Lenteren, 1976; Van Lenteren et al., 1976).

From both experiments we may conclude that *Aphidius* discriminates between parasitized and unparasitized hosts. The behavioural indications for this are as follows. An unparasitized host is first touched by the antennae of the wasp after which stinging (with or without egg deposition) follows. A parasitized host is rejected after the antennal test or after a probe with the ovipositor (during stinging). The host is marked externally, and perhaps also internally although we are not sure whether the ovipositorial test is ex- or internal.

As soon as most hosts are parasitized the wasps leave the leaf and try to fly away.

Functional response

Solomon (1949) defined the functional response as 'a change in the number of prey (host) attacked in a fixed period of time by a single predator (parasite) when the initial prey (host) density is changed'. We tested the functional response of *Aphidius* to see whether this parasite shows an increase in percentage parasitization when host density increases. If so, the parasite has a greater control possibility than one for which such a response is not found. The kind of response curve where the percentage attack increases over a certain range has been described by Holling (1959) as an S-shaped functional response. Up to some years ago such a curve was only found for vertebrates, but since two years (Van Lenteren & Bakker, 1976; Hassell et al., 1977) this kind of response was discovered in invertebrates. This phenomenon was not found earlier because of an unsuitable experimental set-up, in which alternative hosts and/or an opportunity to emigrate was lacking, and the lowest host densities used were too high.

Although these considerations were taken into account at the start of this experiment, it was difficult to determine a proper set-up for testing *Aphidius*' functional response because so little was known about the wasp's biology.

We tested the attack rate of *Aphidius* by placing an individual wasp

together with 0, 1, 2, 4, 8, 16 and 32 aphids on a paprika leaf. On this leaf a perspex ring was put with an inside diameter of 5.5 cm (surface 23.76 cm^2). The wasp was continuously observed for 20 minutes. Per density 8 to 15 tests were done, and for each test a new female was used. Because the wasp was confined ^{to the leaf part} and we observed the animal continuously, we knew the moment and number of escape attempts, i.e. the moment at which the wasp started walking on the perspex ring and on the piece of glass.

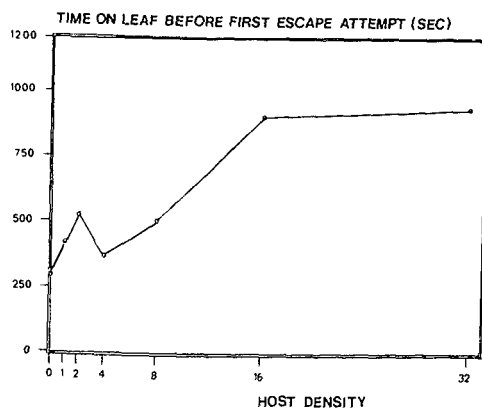


Fig. 3. Time spent on the leaf between introduction moment and the first attempt to escape against host density.

In Fig. 3 the first escape attempts are plotted against density. At a density of zero the wasps tried to escape after on the average 294 seconds, at a density of 1 after 415 seconds, whereas their first attempt to escape at higher densities was after a much longer period (on the average 935 seconds at a density of 32; 9 out of the 14 wasps tested stayed with the hosts for the full 1200 seconds at this density!).

The wasps could not escape, however, and the number of contacts at low densities thus increased due to repeated visits to the leaf. Eventually this resulted in a high percentage superparasitization at low host densities (Fig. 4). This high percentage of superparasitization does not mean that the wasp possesses a weak ability to discriminate. Due to a mounting oviposition tendency, the wasp eventually lays more than one egg per host after frequent rejections.

Fig. 5 shows the number of contacts (with antennae and ovipositor) at different densities, and fig. 6 the number of stings, both only for the first 300 seconds of the experiment. We took this limit of 300 seconds to prevent a strong influence of the repeated visits to the leaf at low host densities. Both the number of contacts and the number of stings increase fast with an increase of hosts at the lower densities.

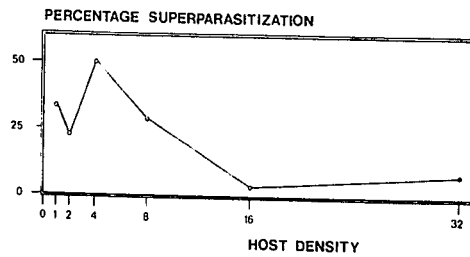


Fig. 4. Percentage superparasitization at different host densities.

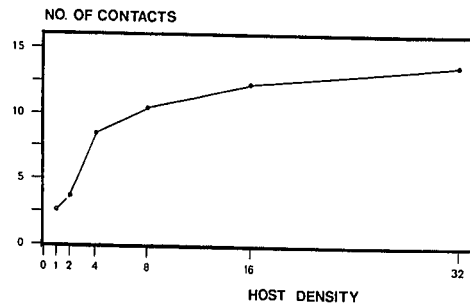


Fig. 5. Number of contacts between parasite and host at different host densities, during the first 300 seconds of the experiment.

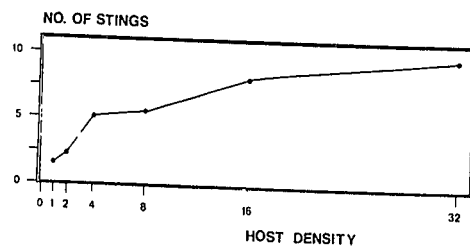


Fig. 6. Number of stings with the ovipositor at different host densities during the first 300 seconds of the experiment.

The number of contacts is, however, already so high at the low host densities, that we must conclude that these low densities were not sufficiently low to test the functional response of *Aphidius* properly.

When we look at fig. 7 we see a steady increase in the number of parasitizations with increasing host density; the percentage parasitization decreased; the highest percentage parasitization is being obtained at the lowest densities (Fig. 8)

From the functional response experiment we can conclude that
 1. the wasp easily detects hosts at a density of 1 per 23.76 cm²; after

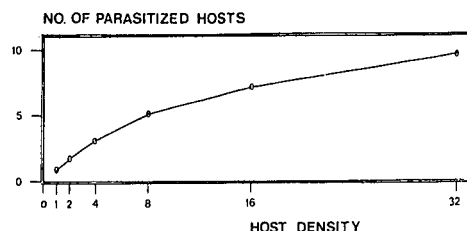


Fig. 7. Number of parasitized hosts at different host densities during the first 300 seconds of the experiment.

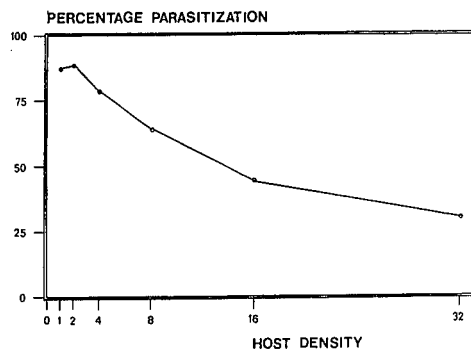


Fig. 8. Percentage parasitization at different host densities after 300 seconds of the experiment.

- only 100 seconds 75% of the hosts were parasitized at this series,
2. the wasp leaves a site where she parasitized (most of) the larvae; emigration is initiated through contacts with parasitized hosts,
3. at high host densities the wasp stays longer on the leaf than at low densities; at the highest density (32) the wasps parasitized 57% of the hosts within 20 minutes, showing a high oviposition frequency, and
4. in order to test the functional response of *Aphidius* properly, lower host densities have to be used.

The same kind of functional response experiments are now being done on intact plants in which the wasps are given an opportunity to emigrate.

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Zusammenfassung

Biologische Merkmale von Aphidius matricariae Hal. (Hymenoptera: Braconidae) einer Parasiten der Grünen Pfirsichblattlaus Myzus persicae (Sulzer).

Die Möglichkeit Aphidius matricariae ein zu setzen zur biologischen Bekämpfung von Myzus persicae an Paprika in Gewachshäusern wird untersucht.

Es stellte sich heraus, dass die Wespe die Larvalstadien der Blattlaus nicht von einander unterscheidet, sondern alle belegt. Verschwendung von Eiern wird verhindert durch die Fähigkeit der Schlupfwespe noch nicht parasitierten von schon parasitierten Blattläusen zu unterscheiden.

Schliesslich zeigte sich, dass die Anzahl der Eiablagen pro Zeiteinheit bedingt ist von der Blattlausdichte.