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Searching behaviour of the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera : Aphidiidae) in response to honeydew excreted by aphids on different host plants

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Abstract. Aphidius rhopalosiphi is a parasitoid, which parasitizes the aphid *Rhopalosiphum padi* on wheat and is a potential candidate in Biological Control Programmes. The effect of honeydew excreted by host aphids *A. rhopalosiphi* and *Metapolophium dirhodum*, and honeydew extracted by non-host aphids, *Mizus percicae* and *Brevicoryne brassicae* on searching and walking time of *A. rhopalosiphi* was tested. Water was used as the control. Walking time of *A. rhopalosiphi* on honeydew treated area was also investigated.

When parasitoids were released, the time taken to reach the honeydew treated discs was significantly shorter than the time taken, to reach the control discs ($P \leq 0.05$). The time taken by parasitoids to reach areas treated with honeydew of different aphid species did not differ significantly among the species ($P \geq 0.05$). The time spent on different honeydew treated discs was significantly higher than controls ($P \leq 0.05$). The time spent on non-host honeydew was significantly shorter than the time spent on the host honeydew ($P \leq 0.05$). Speed of movements of *A. rhopalosiphi* on treated area was significantly higher than non-treated area ($P \leq 0.05$). These results revealed that honeydew of both hosts and non -hosts act as an attractant. Distinct behaviour pattern suggests that host honeydew act as a stimulant as well.

Keywords: Aphidius rhopalosiphi, searching behaviour, host honeydew, non-host honeydew

1. Introduction

Aphids (Hemiptera: Aphidae) are one of the major groups of insect pests of wheat (Emden, 1972). *Aphidius rhopalosiphi* L. (Hymenoptera: Aphidiidae) is a well-known parasitoid of the aphid *Rhopalosiphum padi* (Hemiptera: Aphidae) which feeds on wheat.

Biological control has drawn the attention of scientists as well as policy makers (Martin, et al., 2003) as an alternative to chemical control that brings about harmful effects on the environment as well as on human health. Therefore, it is very important to investigate the searching behaviour of a parasitoid to find the host. Searching time of a parasitoid is one of the indicators of its performances (Vinson, 1978). The olfactometer studies revealed (Wickremasinghe and Van Emden, 1992) that several species of aphid parasitoids have been attracted to the odour of the plant which is host of their aphids, more than to the odour of host aphid or their honeydew, in the absence of the host plant. However, a combination of host plant and host aphid odour proved the most attractive stimulus, of those tested. Although not tested directly the magnitude of the responses suggests that honeydew was a stronger attractant than host aphids. Honeydew is excess sap excreted by aphids following feeding on the host plant phloem sap. The behaviour of parasitoids on honeydew contaminated plants suggested that honeydew could be an arrestant as well as a searching stimulant. An arrestant may be defined as a chemical that congregates insects as a result of undirected kinetic reactions, either the slowing down or stopping locomotion (Orthokinesis) (Shorey, 1977). To investigate this further, effect of honeydew from host and non-host aphid species on searching and walking times of the parasitoids were investigated under the laboratory conditions.

2. Materials and Methods

2.1 Aphids and host plants

The four different aphid species and their host plants selected for the study were *Metapolophium dirhodum* and *Rhopalosiphum padi* found on wheat, *Brevicoryne brassicae* found on Brussels sprouts and *Myzus persicae* found on tomato plants. Each aphid species was reared on the relevant host plant. Plants were kept in culture cages (25×35×60 cm) covered in muslin. Wheat, brussels sprouts and tomato were grown in separate pots (12 cm diameter) and were infested with the relevant aphid species.

2.2 Collection of honeydew

To collect honeydew 10 aphids from each species were caged separately on leaves of its host plant in para film lined clip cages for 24 hours. Honeydew excreted by each aphid species was collected on para film and was removed using capillary tubes. Immediately after collection 2μl of honeydew was applied on to para film discs (3.5 cm diameter) and placed inside a Petri dish. A hole of 1.5 diameter was made in the center of the lid of each Petri dish and a perspex tube (1.5 cm wide and 6 cm long) was fixed to make a passage for introducing parasitoids in to each test chamber.

2.3 Rearing of *Aphidius rhopalosiphi*

Aphidius rhopalosiphi was reared on *R. padi* infesting the wheat variety Hobbit. Wheat was grown in two separate cages (25×35×60 cm). Parasitized aphids were collected using a wet paint brush and placed in specimen tubes lined with cotton wool to prevent desiccation of the parasitized aphids. Parasitoids, 24-48 h after emergence, were used in this experiment.

Behaviour of parasitoids was observed in an observation chamber. A Petri dish containing a honeydew-treated para film disc was placed inside the observation chamber. The top and the four sides of this chamber were covered with opaque oil paper to prevent entry light which could disturb the behaviour of parasitoids. Experiment was started by releasing a single parasitoid at the top of the tube leading to Petri dish in the test chamber. A parasitoid was released by inverting the specimen tube containing the parasitoid over the opening of the central tube. Time taken by parasitoids to reach the honey dew treated disc and the time spent for searching inside the honeydew treated disc was recorded. Ten parasitoids were allowed to search on each honeydew treated para film disc. Eight replicates were used for each treatment. Discs treated with 20 µl of water were used as controls.

In another experiment, whether the honeydew acts as a searching stimulant was tested. The movement of *A. rhopalosiphi* was observed in the treated para film discs. Twenty microliters of honeydew solution was applied to a 4 cm diameter spot in 0.5µl droplets. Treated discs were air dried before use. Eight such discs were prepared. At the beginning of the experiment a Petri dish with a honeydew-treated filter paper was placed inside the observation chamber and a single parasitoid was released into the arena, and the behaviour was observed. The movement of the parasitoid was measured by tracing the path followed by the parasitoid on the filter paper. A transparent paper was placed above the top of the Petri dish and trace of the path was marked directly on the transparent paper. The time spent outside and inside honeydew treated area by each parasitoid was measured and the distance travelled was measured using the trace.

Ten parasitoids were allowed to search on treated discs. Eight treated discs were used in each experiment.

3. Results

The time taken since the release of the parasitoid into the tube above the arena to reach the treated part of the test disc was significantly shorter with honeydew treated than the control ($P \leq 0.05$) To water treated discs 44% of the parasitoids tested failed to react at all. The time taken by parasitoids to reach areas treated with honeydew produced by different aphid speciesdid not differ significantly ($P \leq 0.05$) (Table 1).

Table 1 : Time spent on searching on host/non host honeydew by *A. rhopalosiphi*

Honeydew producing spp.	Time spent on searching X ± SE(s)	Time taken to reach the test disc X ± SE(s)	% parasitoids reached the test chamber
R. padi on wheat	39.342 ± 2.14^{c}	42.93 ± 7.65^{a}	100

M. dirhodum on wheat	36.56 ± 2.15^{c}	44.65 ± 7.47^{a}	100
M. persicae on tomato	14.03 ± 1.24^{b}	51.87 ± 6.95^{a}	100
B. brassicae *on brussels sprouts*	15.34 ± 1.43^{b}	48.84 ± 5.57^{a}	100
Control (water)	9.22 ± 0.76^{a}	181.81 ± 28.3^{b}	56

Means with different supper script letters in each column are significantly different (One way ANOVA using log transformed data). Means separated by LSD at $P \leq 0.05$.

Searching time of a parasitoid is a good indicator of its performances. However, the time spent for searching on honeydew of *R. padi* and *M. persicae* fed on wheat were similar and significantly shorter than the time spent searching on the honeydew of *B. brassicae* fed on Brussels sprouts and *M. persicae* on tomato ($P \leq 0.05$). The speed of movement of *A. rhopalosiphi* inside the honeydew treated area was significantly lower than the out side ($P \leq 0.001$) (Table 2).

Table 2 : Rate of movement of *A. rhopalosiphi* in the presence of honeydew

Location	**Movement (mm/s)**
a) Outside the treated area	14.14 ± 0.31^{a}
b) Inside the treated area	3.24 ± 0.47^{b}

Means followed by different supper script letters are significantly different at $P \leq 0.05$.

When a parasitoid was introduced in to the tube leading to the arena it did not immediately orient itself to the arena containing honeydew but took some time to walk in to the arena. Parasitoids walking towards the area appeared excited, probing the substrate with the antennae as they walked. The time taken by the parasitoid to reach the honeydew treated disc and their behavior inside the inverted tube was similar regardless of the type of honeydew. In the control, parasitoids were not active; many of them remained on the top of the inverted specimen tube without moving out. Only 56% walked inside the tube, but even those took about four times longer duration to reach the water treated compared to honeydew treated discs. All parasitoids responded positively to the honeydew treated discs, indicating the presence of an attractant.

The parasitoids searched for discs treated with honeydew of their hosts (*R. padi* and *M. dirhodum* feeding on wheat) took about half a minute. Abdominal protractions and frequent antennation were observed. Some of the parasitoids tried to oviposit on the honeydew droplets. When parasitoids searched discs with the honeydew of *M. persicae* and *B. brassicae* fed on tomatoes and Brussels sprouts respectively, frequent antennation could be observed but abdominal protractions were rarely observed. This suggests that the host honeydew could be a stimulant most probably for oviposting.

4. Discussion

From the observations and responses measured, it is apparent that *A. rhopalosiphi* responds to odour of non-host and host honeydew and could discriminate host from non host honeydew upon contact. The time spent searching discs with honeydew of *R. padi* and *M. dirhodum* was about 2.5 times greater than the time spent searching with honeydew of *M. persicae* and *B. brassicae*.

In the second experiment, parasitoids walking across the untreated area showed distinct behaviourial changes upon contact with the honeydew contaminated area. When parasitoids were released on to the test disc, firstly they walked rapidly; did not show any abdominal movements or antennation and they rarely turned back. However, when it entered the honeydew treated area, frequent abdominal protractions and zig- zag movements (angle turns) increased. Parasitoids stopped very frequently and touched the substrate with the antennae. Parasitoids moving out of honeydew treated area touched the filter paper with antennae and quickly turned back towards the area. Apparently the overall retention of parasitoids on honeydew is reflected through several different mechanisms shown by the parasitoids as arrest, decreased walking speed (orthokinesis) and increased turnings (klinokinesis). Honeydew therefore appears to be an arrestant, which keeps the parasitoid in the honeydew contaminated area, increasing the chance of contact of host aphids.

The attraction of *A. nigripes* toward both non-host and hosts aphids has been reported by Bouchard and Cloutier (1984). *A. nigrips* responded positively to the odour of the honeydew of the preferred host *Macrosiphum euphobiae* but also that of less preferred hosts such as *Myzus perssicae, Apis nasturtii* and a non host *Rhopalasiphum maidis.* Hagen *et al*. (1976) reported that *Chrysopa carnea,* a predator of *Theiroaphis trifolii* was attracted to the odour of the host honeydew; van Emden and Hagen (1976) subsequently found that a break down product of the tryptophan in the honeydew was the attractant, which Hagen *et al.* (1976) reported as sucrose the arrestant. Behavior of *A. rhopalosiphi* suggestes that attractant may be present in both host and non-host honeydew, but the stimulant is present only in host honeydew. Frequent abdominal protractions by *A. rhopalosiphi* at host honeydew indicated that an oviposition stimulant may be present in the honeydew and this response would cause the parasitoid to increase its searching time. That abdominal protractions were rare on non-host honeydew indicated that the oviposition stimulant was either absent or most probably present in smaller amounts. Such differences between host and non-host honeydew might be expected to arise from diversity of chemicals present in the honeydew of aphids feeding on different plants (Auclair, 1958, 1963; Sidhu and Patton, 1970).

Similar changes in the locomotory behaviour following contact with kairomones have been found for other parasitoids (Chiri and Leghan, 1982; Strand and Vinson (1982); Vet and van der Hoven, 1984; Waage, 1978). Strand and Vinson (1982) reported increased walking speed of *Cardiochiles nigriceps*. A behavioural response similar to that shown by *A. rhopalosiphi* in this study, has been reported by Waage (1978) for locomotory response of the ichneumoid *Nemeritis canescence* to the mandibular gland secretion of its host *Plodia interpucntella*. In response to the presentation of the host chemical on a surface, a walking *Nemeritis* exhibited a complex orthokinetic responses involving stopping, walking at a reduced speed and probing with the ovipositor. The wasps loosing contact with the treated area exhibited a klinokinetic response and returned back to the treated area. Two *Leptopilina* species such as *L. fembriata* and *L. heterotoma*, which attacks *Drosophila* spp. reduce their walking speed and increase their probing frequency (Chiri and Lengner, 1982). A significant increase in the number of both oviposition reactions (host stinging) and egg laying has been observed in *Aphidius eivi* when the dummies of aphids are coated with conical secretion (Larocca, 2007). However this enhancement has not been observed when the aphid dummies contained distilled water. Budenburg & Powell (1997) has reported that *Episyrphus balteatus* (Deg) landed more frequently on wheat ears contaminated with host aphid honeydew than on clean ears suggesting a response to honeydew volatiles. The number of eggs laid by *E. baltetus* has been increased with increasing honeydew concentration.

Honeydew of host aphids attracts the parasitoids from a distance (Bouchard & Cloutier, 1984) then arrests the parasitoid in the contaminated area. Slowing down the increased antennae examination would increase the possibility of the parasitoid encountering the host. The klinokinatic response would increase intense searching throughout the contaminated area, further increasing the chance of finding host.

References

Auclair, J.L. (1958). Honeydew excretion in the pea aphid *Acyrthosiphon pisum* (Homoptera; Aphiidae) J. Insect Physio.2, 330-337.

Bouchard, and Clouher, (1984). Honeydew as a source of host searching kairomone for the aphid parasitoid *A. negriceps* (Hymenoptera; Aphidiodae). canad. J. 2001. 62, 1513-1520.

Bouch, G.M., Gaerawald, R.L. & Miyasaki, S. (1969). Development of a chemically defined diet for adults of the apple maggot based on amino acid analysis of honeydew. Ann. Ent. Soc. Am. 62, 19-21.

Budenburg, W.J. and Powell, W. (1997). Ent. Exp. Et Appl. 64, 57-61.

Chiri, A.A. & Leghan, E.F. (1982). Host searching kairomones alter behavior of *Chelonus* sp., a hymenopterous parasite of the pink bollworm, *Pechinophora gossypiella* (saunders) Environ. Ent 11, 452-455.

Emden V. H.F., Hagen, K.S. (1976). Olfactory researches of green lacewing, *Chzysopa carnea* to tryptophan and certain breakdown products. Environ. Ent. 5, 469-473.

Hagen, K.S., Shawall, E.F. and Tassan, R.L. (1976). Tryptophan in artificial honeydew as a source of an attractant for adult *Chzysopa cavaea*. Environ. Ent. 5, 458-468.

Larocca, M (2007). Functional bases of host acceptance behaviour in the aphid parasitoids *Aphidius ervi*. Physiol. Ent. 32, 305-312.

Martin, H, Schmidt, Andreaslaeur, Tobias and Carlsten Thies (2003). Relative importance of predators and parasitoids for cereal aphid control. Environ. Ent. 270, 1527, 1905-1909.

Sidhu, H.S., & Patton, R.L. (1970). Carbohydrates and nitrogenous compounds in the honeydew of the mustard aphid, *Lipaphis ezysimi*. J. Inset physiolo. 16. 1339-1348.

Shorey, H.H. (1977). Interactions of insect with their chemical environment. In chemical control of Insect behavior, Edited by H.H. Shorey, J.J. Shorey & J.J. Mckelvey. pp1-5. Wiley, New York.

Strand, M.R. & Vinson S.B. (1982). Behavioral responses of the parasitoid *Casdiochiles nigriceps* to a kairomone. Ent. Exp. Appl. 31 308-315.

Vet, L.E.M. & Van Der Hoeven, R. (1984). Comparison of the behavioral response of two *Leptopilicina* species (Hymenoptera; Eucoilidae) living in different microhabitats, to kairomone of their host (Drosophilidae). Neth. J. 2001. 34 220-227.

Vinson, S.B., Haclan, D.P.& Hart, G.W. (1978). Responses of the parasitoid, *Microtestes flavus* to the brown spot scale and its honeydew. Environ. Ent.7, 874-878.

Waage, J.K. (1978). Arresment responses of the parasitoid, *Nemuritis canescens*, to a contact chemical produced by its host, *Plodina intelpunctella*. Physiolo. Ent. 3, 135-146.

Wickremasinghe, M.G.V & Van Emden, H.F. (1992). Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi,* to volatile chemical cues from the host plants of their aphid prey. Physiolo. Ent.17,297-304.