EVALUATION OF HOVERFLY *EPISYRPHUS BALTEATUS* DE GEER (DIPTERA: SYRPHIDAE) OVIPOSITION BEHAVIOUR TOWARD APHID-INFESTED PLANTS USING A LEAF DISC SYSTEM

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SUMMARY

Several aphidophagous beneficials such as parasitoids and predators are known to respond positively to aphid infested plants. Semiochemicals from the latter association play an important role in the foraging of predators in a tritrophic approach. In this work, three host plants infested with green peach aphid, *Myzus persicae* Sulzer were used to study the effect of prey density and aphid colony location on plant toward *Episyrphus balteatus* female. Their reproductive behaviour and efficiency (in terms of fecundity) were observed in net cages. Three kinds of experiments were performed using a disc leaf on agar diet in small Petri dishes under different conditions: 1) leaf disc of broad bean (*Vicia faba*) were infested with different aphid prey densities, 2) three host plants (*Vicia faba, Solanum tuberosum, Solanum nigrum*) infested with 3 different aphid prey densities and 3) three different heights of aphid colony location on *V. faba* plant infested with constant aphid density were also tested using leaf disc system. Aphid-free leaf discs were also used as control for predator behaviour observations. Oviposition rates of hoverfly on leaf disc system vary significantly with prey aphid densities. The means of eggs per laying were 0.9, 5.3, and 31.2 for 0, 10 and 100 aphid densities respectively. *E. balteatus* females were also able to evaluate and adjust oviposition rates according to different aphid prey densities/host plant. The 5 and 20 cm stakes were the most attractive heights of aphid colony location for the hoverfly oviposition. The means of eggs per laying were 16.7, 18.5 and 5.8 for 5, 20 and 40cm heights respectively. Our leaf disc system was found to be a practical and efficient way to assess chemical cues from aphids according to different conditions on the hoverfly reproductive behaviour.

Key words: *Vicia faba*, *Myzus persicae*, Semiochemicals, *Episyrphus balteatus*, Leaf disc system, Oviposition behaviour.

INTRODUCTION

Predator responses toward variation of different prey density have been a central theme in ecology theory (Hassell and May, 1974; Kareiva and Odell, 1987) and biological control (Beddington *et al.*, 1978; Murdoch *et al.*, 1985). A predator that responds numerically to increasing size pest colony and oviposits on plants with higher prey density is thought to be ideal for suppressing pest populations before they reach damaging levels (Murdoch *et al.*, 1985; Waage and Greathead, 1988; Murdoch and Briggs, 1996).
In a tritrophic approach the semiochemical cues emitted from aphids may be attractive for the natural enemies. For example, honeydew produced by the cereal aphid *Stibion avenae* (F.) plays an important role as effective contact

kairomone for the ladybirds *C. septempunctata* and *Adalia pipunctata* (Casing and Dixon, 1984, Al Abassi *et al.*, 2000; Francis *et al.*, 2004a). Also, the honeydew of the pea aphid *A. pisum* stimulates the egg laying by *E. balteatus* (Budenberge and Powell, 1992). In aphidophagous hoverflies that are effective natural enemies of various aphids, Chandler (1968a) divided aphidophagous species into two categories: species in which the aphid is the oviposition stimulus are termed "aphidozetic", while the other species for which the plant stimulus is dominant are called "phytozetic". The close relationship between the ovipositional selection of hoverflies aphidophagous "aphidozetic" and the colony size or density of aphids has been observed (Dixon, 1959; Ono, 1962; Bombosch 1962, 1963; Volk 1964; Chandler, 1965; Chandler, 1968a, b; Ito & Iwao, 1977). In particular, the hoverfly *Episyrphus balteatus* is a member of the "aphidozetic" group (Chandler 1968a). Female flies and lays its eggs near aphid colonies (Chandler, 1968 ab; Tenhumberg and Poehling, 1995; Scholz and Poehling, 2000). The study reported here aims to investigate oviposition behaviour of hoverfly *E. balteatus* in response to different aphid prey densities and also to different heights of aphid colony location using a leaf disc system and broad beans plants.

MATERIALS AND METHODS

Plant and insects rearing

Broad beans (*Vicia faba* L.) were grown in 30×20×5 cm plastic trays including a mixture of vermiculite and perlite (1/1) and were used as host plants to rear *Megoura viciae* Buckton and *Myzus persicae*.
Each aphid species and syrphids were reared in a controlled environment room at 20±2°C, under a L16: D8 photoperiod. Adult hoverflies were reared in 75×60×90 cm cages and were provided with bee-collected pollen, sugar placed on petri-dish lids on the floor of the cage, and water. Bean plants infested with *M. viciae* were introduced into the net cages for 3h every third days to allow oviposition. Hoverfly larvae were mass-reared in aerated plastic boxes (110×140×40 mm) and were daily fed *ad libitum* with *M. viciae* as standard diet.

Preparation of leaf disc system

The leaf disc system was made of three components: (1) Leaf disc from of broad bean (*V. faba*), (2) aphids, (3) agar diet (agar 1% solution w:w) prepared in a small petri dish (25 mm diameter). The system was positioned on stakes made of plexiglas placing the leaf disc system at a height of 20 cm (Figure 1A).

Oviposition behaviour of adult syrphids

Oviposition behaviour of *E. balteatus* toward aphid infested plant was investigated in three kinds of experiments using leaf disc system and healthy plant.

Experiment 1. Influence of different aphid densities on syrphid oviposition

This experiment was carried out with two tests:
In the first test, three (3) different densities (0, 10 and 100 individuals) of *M. persicae* were presented simultaneously to the hoverfly female in net cage and allowed to oviposit for 3h before being removed (Figure 1).
In the second test: *V. faba* plants were grown individually in 10 cm diameter plastic pots including a mixture of vermiculite and perlite (1/1; V/V). Those plants (stem with 6 true leaves, 20 cm high) were infested with the same three different densities of *M. persicae* (0, 10, and 100 individuals) and tested as control in order to compare each systems. A single female was introduced and allowed to oviposit for 3h.

Experiment 2. Influence of host plants infested with different aphid densities on syrphid oviposition

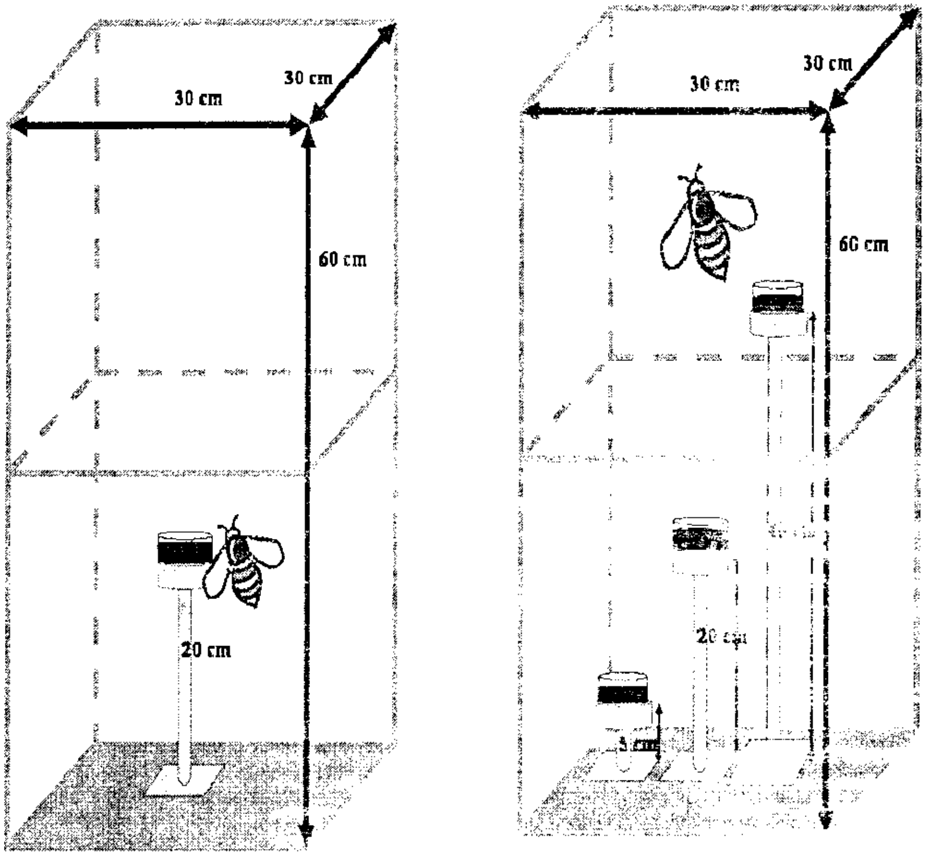
M. persicae were reared for several generations on three different host plants: namely bean (*V. faba*), potato (*Solanum tuberosum*) "Binch" variety and *Solanum nigrum*). Three different densities (25, 75 and 125) of *M. persicae* ou each host plant were presented in a randomized sequence to each syrphid female on stakes made of plexiglas as supports to leaf disc system at height 20 cm (i.e, a no-choice situation, with only one aphid density available at any time) (Figure 1A). Each presentation of 3 different densities of *M. persicae*/host plant lasted for 3h. Every two days, females had the choice to oviposit on the three different aphid densities of each host plant in net cage (30×30×60 cm), in a controlled environment room at 21±1 C°, under a L16: D8 photoperiod (n= 6 replicates).
In this experiment, the syrphid oviposition was also estimated using three entire host plants infested with constant number of *M. persicae* (400 individuals) for 3h as control for comparing with the syrphid oviposition on leaf disc system.

Experiment 3. Influence of aphid colony location at different heights on syrphid oviposition

Three heights (5, 20 and 40 cm) of aphid colony location were tested on stakes as supports for leaf disc system infested with constant density of *M. persicae* (125 individuals) using three-choice experiment. Indeed, single female was offered simultaneously a choice between three different heights of aphid colony location in net cage (30×30×60 cm) (Figure 1 B).
Tested *E. balteatus* females were 20 days old and had been deprived of oviposition sites for 24h prior to testing. A single female was introduced into the net cage and allowed to oviposit for 3h before being removed. The number of eggs laid on each leaf disc/density of aphid was then counted (10 replicates per experiment).

STATISTICAL ANALYSIS

All mean comparison tests were realised using the SAS program (1998 version) and by performing analysis of variance, (General Linear Models). The mean numbers of eggs laid by females on leaf disc system according to different densities were compared using Duncan's multiple range test.



(A) (B)

Figure 1. Illustration of leaf disc system used to assess the fecundity behaviour of *E. balteatus* toward aphid colony size on single height (A), and location on different heights (B)

RESULTS

Influence of different aphid densities on syrphid oviposition

The results of three – choice experiment (3 densities of *M. persicae* infested leaf disc) and one-choice experiment (3 densities of *M. persicae* infesting *V. faba* plant) are presented in (Figure 2A, B) respectively: oviposition rates on leaf disc system with high aphid density infestation (100 individuals) was significantly higher from those on leaf disc system with free and low aphid densities (0, 10) (P<0.001). Moreover, egg deposition by *E. balteatus* female was also density dependent using aphids infested bean plants (*V. faba*)

Higher significant differences between the numbers of eggs laid by *E. balteatus* on bean plant infested with high aphid density (100 individuals) and those with free and low aphid density (0, 10) ($P<0.05$). We observed consequently, the results obtained when the syrphid fecundity was evaluated by leaf disc system corresponded to those obtained using natural entire bean plant *V. faba* infested by aphids (see Table 1).

Influence of host plants infested by different aphid densities on syrphid oviposition.

The host plant infested with different densities of *M. persicae* (25, 75 and 125) using the leaf disc system significantly influenced the number of eggs laid by the hoverfly female ($P<0.05$) (Figure 3A, B). According to aphid colony size infested host plant, there is no significant difference between all three host plants (*V. faba*, *S. tuberosum* and *S. nigrum*) in terms of fecundity. Similar results have also been obtained when comparing the number of eggs laid by *E. balteatus* female on leaf host plant disc infested with 125 aphids and entire host plants infested by 400 aphids (see Table 2).

Influence of aphid colony location on syrphid oviposition.

In the three – choice experiment (3 heights of *M. persicae* colony positioned on leaf disc system), the mean of eggs laid by *E. balteatus* on *M. persicae* colony positioned on leaf disc at 5 and 20 cm heights were significantly higher from those on leaf disc at 40 cm height ($P<0.05$) (Figure 4). In this study, after the investigation of hoverfly behaviour fecundity using a leaf disc system during the first, second and third experiments, the leaf disc bioassay was shown to be an efficient method to estimate the oviposition behaviour of *E. balteatus*.

DISCUSSION

The observations of the relationship between the oviposition behaviour of aphidophagous syrphid flies and aphid colony size in the field (Dixon, 1959; Tenhumberg, 1993) and net cage (Ito and Iwao., 1977; Geusen-Pfister, 1987; Bargen *et al.*, 1998; Scolz & Poehling, 2000; Sutherland *et al.*, 2001) demonstrated that the numbers of syrphid eggs on a plant were found to be positively correlated with aphid densities. In our experiments, *E. balteatus* female demonstrated a positive density-depended response to aphid colony size in terms of oviposition behaviour using leaf disc system and bean plant.

The suitability of an oviposition site does not only depend on the number of aphids present at the time of oviposition, but it has also been depended on the quality of the aphid colony (Kan & Sasakawa, 1986, Kan, 1988a,b). It is possible that females avoid very high density aphid colonies, since such colonies are subject to increased emigrating of the prey (Kan, 1988a,b), and therefore may not support the full development of several syrphid larvae. In adition, there are others factors may help also to determine the oviposition rates of syrphid female, for example, the chemical cues emanating from host plant infested aphid may be elicite the oviposition hoverfly (Dixon, 1959, Bombosch and Volk 1966). In our study we found that the densities of aphids themselves did not only stimulate the eggs laid by *E. balteatus* female, but also the quantity of oviposition-eliciting substances emanating from the aphid colonie size associated with different host plants because the results demonstrated that the oviposition rates of *E. balteatus* increase in response to different aphid densities infested different host plants using leaf disc systems.

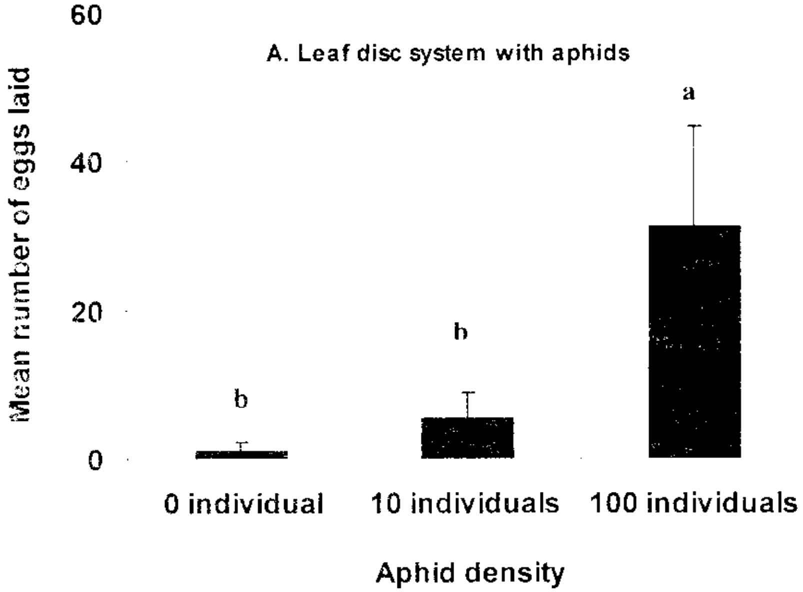
The height preference of various syrphid species was already related to their habitat preferences. Indeed, Chandler (1968) demonstrated that univoltine syrphid species of that developing in spring, when aphids are present on trees and shrubs but are rare on herbaceous plants, tended to oviposit around 180 cm. All syrphids species that develop in early summer, when aphids are abundant on herbaceous cover, tended to oviposit at height 30 cm. Those that are abundant throughout the year showed no strong consistent preferences. In our study, *E. balteatus* changed its egg laying response to different heights of aphid colony location on leaf disc system, laying more eggs on lower height colonies location until 20 cm. In conclusion, our study shown that, leaf disc system on diet agar demonstrated to be a useful and efficient method to assess in the laboratory the biological effect of chemical cues from aphid toward *E. balteatus* behaviour.

Table 1. Comparison of the mean numbers of eggs laid by hoverfly *E. balteatus* in response to different densities of *M. persicae* using leaf broad bean plant disc system and whole broad bean plant

Number of eggs laid by *E. balteatus* female in relation to aphid density		
Aphid density	Leaf disc system with aphid	Aphid infested broad bean plant
0	0.9 ± 1.3	0.0 ± 0.0
10	5.3 ± 3.6	8.5 ± 3.5
100	31.2 ± 13.6	45.0 ± 16.8

Table 2. Comparison of the mean numbers of eggs laid by hoverfly *E. balteatus* on different host plants (*V. faba*, *S. tuberosum* and *S. nigrum*) infested with *M. persicae* using leaf host plant disc system and whole host plant

Mean number of eggs laid by *E. balteatus* female in relation to aphid density		
Host plant	Leaf disc system infected aphid (125 individuals)	Aphid infested host plant (400 individuals)
V. faba	32.2 ± 8.2 a	53.3 ± 32.3 a
S. tuberosum	35.3 ± 15.2 a	48.9 ± 22.4 a
S. nigrum	26.3 ± 5.5 a	33.8 ± 16.8 a



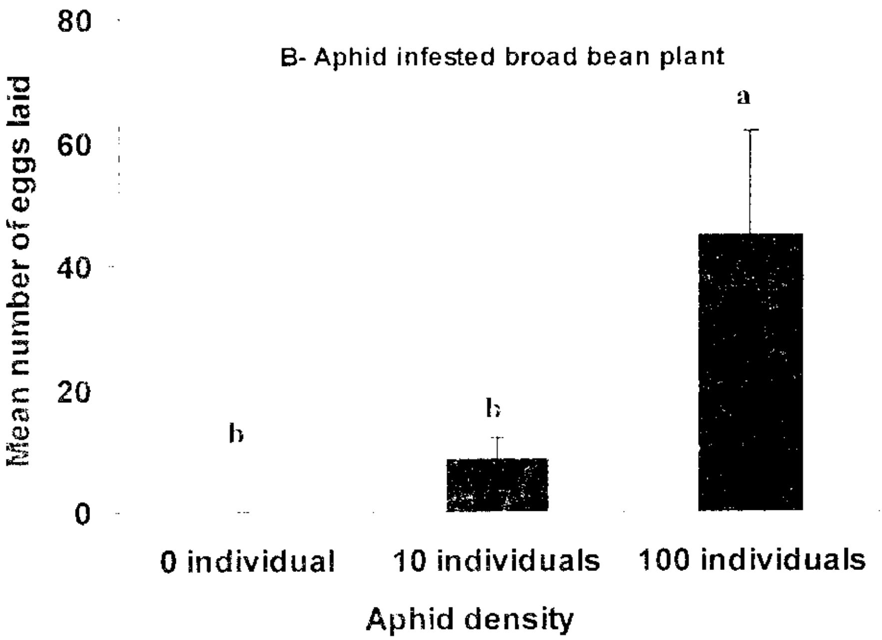


Figure 2. Mean number of *E. balteatus* eggs laid on leaf disc system (A) and whole broad bean plants (B) in response to different densities of *M. persicae* means (±SE) marked with the same letters do not differ significantly (P<0.05)

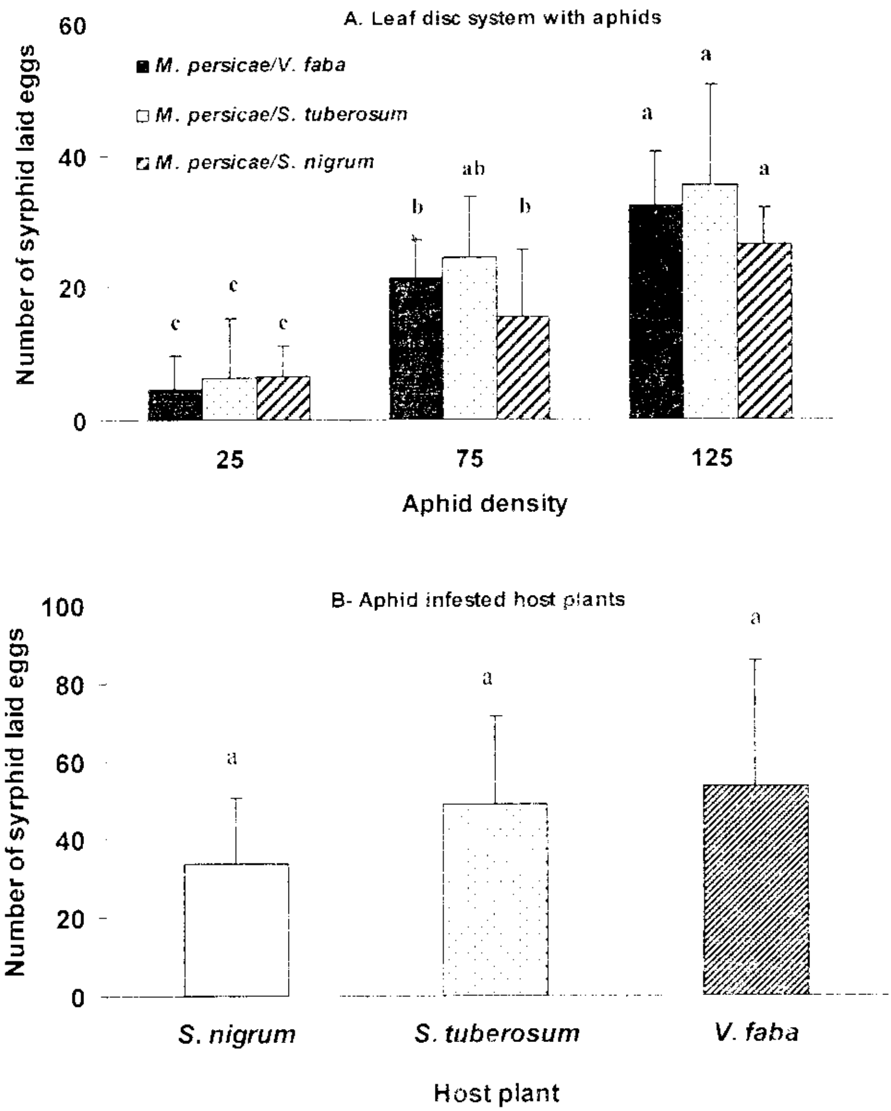


Figure 3. Mean numbers of *Episyrphus balteatus* eggs laid on host plants infested with different densities of *M. persicae* colony size. Different letters indicate significant differences between treatments ($P<0.05$). Bars indicate standard errors of the means.

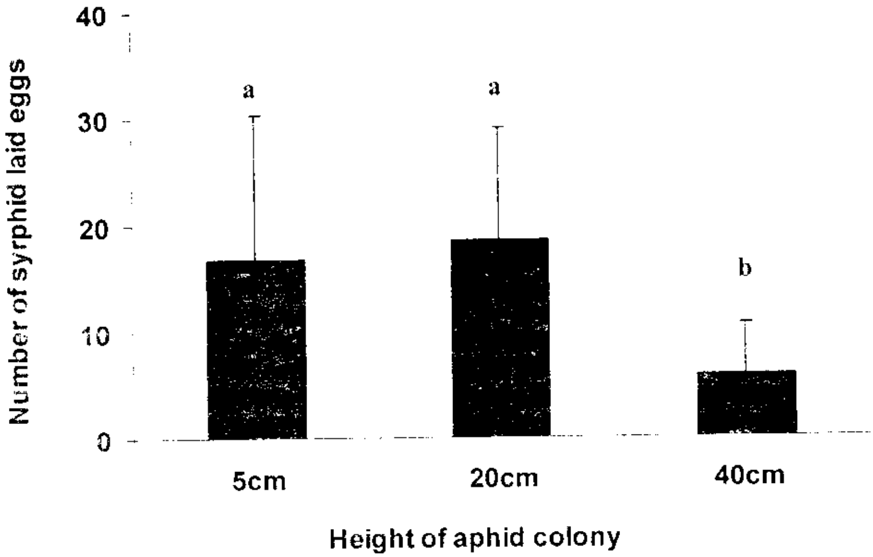


Figure 4. Effect of *M. persicae* aphid colony location on *E. balteatus* oviposition rates means (± SE) marked with the same letter do not differ significantly (P<0.05)

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