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Foraging and oviposition behaviour in the predatory hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae): a multitrophic approach

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Almohamad Raki (2010). Le comportement de recherche et de ponte des femelles du syrphe ceinturé, *Episyrphus balteatus* DeGeer (Diptère, Syrphidae) : approche multitrophique (Thèse de doctorat en anglais). Gembloux, Belgium, Université de Liège – Gembloux Agro-Bio Tech, 165p, 24 fig., 8 tab.

Résumé : Les larves du syrphe aphidiphage *Episyrphus balteatus* (Diptère, Syrphidae) ont une capacité de déplacement limitée. Le choix du site d'oviposition des femelles est donc crucial pour la survie larvaire. Pour cela, les femelles de ce diptère devraient optimiser leurs choix de sites d'oviposition pour maximiser les chances de survie de leurs progénitures. Le but de cette thèse de doctorat était de comprendre comment les femelles d'*E. balteatus* évaluent la qualité de patches de pucerons au cours de leur comportement de ponte. La réponse de ponte des femelles d'*E. balteatus* observées à travers plusieurs facteurs a été réalisée, parmi lesquels: la plante hôte, l'espèce de pucerons, la taille de la colonie de pucerons, les substances sémiochimiques émises par les pucerons et leurs associations avec leurs plantes hôtes, la présence de compétiteurs intra- ou interspécifiques et l'âge de la femelle. Dans la première partie de ce travail, les résultats obtenus ont permis de mettre en évidence que les femelles d'*E. balteatus* sélectionnent le site de ponte en fonction de la plante hôte et l'espèce de puceron, et ce comportement a été aussi montré comme étant lié avec la performance du prédateur (fitness). La combinaison puceron/plante *M. persicae*/*Solanum tuberosum* a été la plus préférée par les femelles d'*E. balteatus*, et le fitness de ce prédateur était plus élevé en particulier lorsque les larves sont nourries avec *M. persicae* élevées sur une plante de pomme de terre. De même, les femelles d'*E. balteatus* ont montré une même préférence pour les deux combinaisons *M. viciae*/*V. faba* et *A. pisum* /*V. faba*, et un fitness supérieur du prédateur adulte a été aussi observé lorsque les larves sont nourries avec les deux espèces de pucerons. De plus, nous avons aussi montré que les femelles d'*E. balteatus* en recherche de site de ponte sont guidées par des substances volatiles, en particulier le E-(β)-farnésène, émises par les pucerons infestant leur plante hôte. Dans la deuxième partie, le système feuille-disque est démontré comme étant une méthode pratique et efficace pour évaluer le comportement de ponte d'*E. balteatus* au laboratoire sous différentes conditions. Les résultats ont aussi démontré qu'il y a une relation quadratique entre l'émission de l'E-(β)-farnésène et la taille de colonie de pucerons, ce qui permet de s'effectuer que cette molécule a un rôle important dans le comportement de ponte des femelles d'*E. balteatus* en réponse à la taille de la colonie de pucerons. Les résultats obtenus dans la troisième partie, nous ont permis de montrer que les femelles d'*E. balteatus* réduisent leurs pontes dans une colonie de pucerons contenant préalablement des larves de leur propre espèce ou leurs traces. Une réponse similaire a été aussi montrée en présence des substrats préalablement visitées par les larves de coccinelle *Harmonia axyridis*. Ainsi, la réduction de la ponte des femelles d'*E. balteatus* est provoquée par des substances volatiles émises par les substrats des larves de syrphe. Nous avons aussi démontré que la présence du parasitoïde *Aphidius ervi* dans une colonie de pucerons a un effet significatif sur le comportement de ponte des femelles d'*E. balteatus*. Les femelles d'*E. balteatus* ne distinguent pas les plants infestées par les pucerons parasités ou non parasités, cependant les femelles réduisent leur pontes en réponse à la présence des pucerons momifiés ou des exuvies de momies. De plus, un fitness supérieur du prédateur a été aussi observé lorsque les larves sont nourries avec l'espèce de pucerons *A. pisum* parasités ou non parasités. Enfin dans la dernière partie, l'âge de la femelle d'*E. balteatus* influence significativement leur reproduction, ce qui permet de proposer que les jeunes femelles (2 à 5 semaines) peuvent être plus efficaces dans la lutte biologique contre les pucerons car elles ont une grande efficacité de reproduction.

Toutes les expériences ont été effectuées au laboratoire et la plupart des résultats obtenus sont discutés en relation avec le contexte de la lutte biologique.

Almohamad Raki (2010). Foraging and oviposition behaviour in the predatory hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae): a multitrophic approach (PhD thesis). University of Liege – Gembloux Agro-Bio Tech, 165p., 24 fig., 8 tab.

Summary: The larvae of predatory hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae), have limited dispersal ability to forage. The selection of the oviposition site by gravid females is crucial for the survival larvae. Therefore hoverfly females should optimise their foraging behaviour by choosing suitable oviposition sites. The aim of this PhD thesis was to understand how hoverfly females assess aphid patch quality during their egg-laying behaviour. The impact of several factors on the oviposition response of *E. balteatus* females including host plant, aphid species, aphid colony size, semiochemicals emitted from aphids or their association with host plants, presence of intra- or interspecific competitors and female's age, was clearly demonstrated during this research. In the first part, we have shown that *E. balteatus* females select their oviposition site according to aphid-host plant and aphid species, which is also shown to be related to offspring performance (fitness). Aphid species *Myzus persicae* (Sulzer) infested-*Solanum tuberosum* L. was the most preferred aphid-plant combination as an oviposition site by syrphid females. The *E. balteatus* survival was enhanced in this system and females laid numerous eggs when larvae were reared with *M. persicae* as prey, especially when the host plant was potato. Broad bean plants *Vicia faba* infested with *Megoura viciae* (Buckton) or *Acyrthosiphon pisum* (Harris) were equally attractive for *E. balteatus* females. *Aphis fabae* (Scopoli) was the least preferred aphid. Higher hoverfly fitness was also observed when larvae were reared on *M. viciae* or *A. pisum* compared to those reared on *A. fabae*. Moreover, it was also demonstrated that foraging hoverfly females is guided by different infochemical cues emitted by aphid host plant, such as (*E*)-β-farnesene, enabling them to locate aphid infested plant and to select an adequate oviposition site. In the second part, the leaf disc system was found to be a practical and efficient method to assess the hoverfly reproductive behaviour under different laboratory conditions. Results also showed that there was a significant quadratic relationship between the released (*E*)-β-farnesene amounts and aphid colony size, which means that this molecule play important role in oviposition decision made by hoverfly females in response to aphid colony size. In the third part, we have highlighted that the *E. balteatus* females avoid aphid colonies in which conspecific larvae or their tracks were already present. Similar response was also shown by females to the presence of *Harmonia axyridis* (Pallas) larval tracks. This oviposition deterring stimulus was also shown to be mediated by odourant cues emitted from larval tracks extracts. It was also demonstrated that the foraging behaviour of hoverfly females was modified by the presence of parasitoids *Aphidius ervi* (Haliday) in aphid colonies. Females did not exhibit any preference for plants infested with unparasitised or parasitised aphids for 7 days, but they are reluctant to lay eggs in response to the presence of mummies or their exuvia on broad bean plants. Oviposition preference of predatory hoverfly females according to the developmental state of the parasitoid larvae in aphid prey was also found to be related with larval performance. Finally, the age of hoverfly females was found to be an important factor affecting their reproduction ability, suggesting that younger *E. balteatus* females (2 to 5 weeks old) could be have potential to play a role in biological control of aphids because of their higher reproductive efficiency.

All experiments were performed in a laboratory environment and most results obtained are discussed in relation to the context of biological control efforts.

I dedicate this work to my Father and mother and I hope that they are satisfied and honored.

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List of publications, posters and oral presentation

The results described in present PhD study were taken from the publications listed below. Most of them were presented through the use of posters or oral presentations

Publications

Almohamad R., Verheggen F., Francis F. & Haubruge E. (2006). Evaluation of hoverfly *Episyrphus balteatus* De Geer (Diptera: Syrphidae) oviposition behaviour toward aphid-infested plants using a leaf disc system. *Communications in agricultural and applied biological sciences, Ghent University*. **71**: 403-412.

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Oral presentation

Almohamad R., Arnaud L., Francis F. & Haubruge, E. (2005). Oviposition behaviour of *Episyrphus balteatus* (Diptera: Syrphidae): effect of host plant and aphid density.

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Chapter 1

General Introduction

Natural resources constitute the basis of our existence on earth. Some of these, like food, pharmaceuticals, clean water, air and fossil energy are obvious, whereas some indirect resource, like plant protection from pest or disease outbreak remain obscure to many of us. This is because the indirect ecosystem effects are emergent properties of complex interactions of species with their biotic and abiotic environment (Levin, 1999).

Plants in nature and agriculture face a diversity of challenges that involve both pathogens and insect phytophages. It has been roughly found that 10% of plants' resources are lost to phytophagous insects in natural ecosystem (Kleijn *et al*., 2001). In agro-ecosystems, it is estimated that pre-harvest crop losses causes by insect pests increased from 35% to 45% in the period of 1965 to 1990 (Pimentel, 1991; Lewis *et al*., 1997). Moreover, estimates of crop losses in developing countries including post-harvest losses may reach 60-70% (Thomas, 1999). Phytophagous insects are economically important in agricultural ecosystems. Among those, aphids are major insect pests of agriculture, horticulture and forestry. They cause damage to a wide variety of crops either directly by feeding damage (phloem-feeding aphids) or indirectly by transmission of plant virus diseases (Van Edmen & Harrington, 2007). Aphidoidea can adversely affect crop yield and quality in a number of ways other than by transmitting viruses. For example, excretion of body wax and honeydew can influence crop quality (Drees & Jackman, 1998). When aphids are abundant, they can excrete large amounts of honeydew that supports the growth of black sooty mould, which is caused by filamentous ascomycetes (e.g. *Capnodium citri*) (Reynolds, 1999). The resulting discoloration of crop products affects plant photosynthesis and significantly reduces their market value. Some aphid species are known to be limiting biotic factors for crop yield when outbreaks occur, while other aphid species have caused profound ecological and sociological impacts. A well-known recent example is the introduction of *Diuraphis noxia* Kurdjumov (Russian wheat aphid) in North America (Quisenberry & Peairs, 1998). Morrison & Peairs (1998) estimated that the economic losses caused by *D. noxia* to cereal crops in the USA were US$893 millions between 1987 and 1993.

These homopterous insects have complex life cycles but during the summer most species reproduce asexually and live as clonal, fast growing colonies. Depending on environmental cues such as day length and temperature, these colonies produce sexual morphs in the autumn when mating occurs. Ten per cent of aphid species are host alternating (heteroecious), moving between woody and herbaceous host plants according to season (Eastop, 1977). Migration between the two different species of host plant clearly requires winged morph production by the colony. Even for non host alternating (autoecious) species, however, wing induction is important, as winged morphs allow a clone to take advantage of several individual host plants during a season when the quantity and quality of the host plants change.

In some aphid species, notably *Myzus persicae* Sulzer, because its rapid evolution of highly resistant forms involving cross-resistance mechanisms (Devonshire, 1989; Foster *et al*., 2000), there is, however, an increasing problem with aphid pests that become resistant to the pesticides that are used (Herron *et al*., 2001; Kift *et al*., 2004). Its resistance to pesticides was first observed in the 1950s and 1960s and is now a global problem, causing massive expenditure on control. The pesticides may also harm non-target organisms and leave chemical residues in the products. Moreover, pesticide-treated crops are less attractive to honey bees, which are needed to pollinate many crops (Kearns & Inouye, 1997). Thereby, by responding to consumer demand, governmental restrictions, and the grower’s concern about their own health, additional major research efforts could be spawned in various countries worldwide to find alternatives to chemical control, particularly biological control. The use of predators and parasitoids should be therefore a primary consideration in any pest management program. Biological control is generally the best method of control on the basis of ecological and environmental considerations. The successes achieved more than half a century ago continue to work to this day. Furthermore, biological control has been especially successful in case of pests that are difficult to control otherwise because of the higher ability of biological control agent (i.e. predator and parasitoid) in searching for host (pest). Nearly 66 % of total successes have been obtained in homopterous insects, which are covered by a waxy layer and are not easily killed by contact insecticides. Another 18 % of successes have been reported in case of Lepidoptera, a majority of which are borers and internal feeders (Dhaliwal & Arora, 2001).

Aphid communities are commonly subjected to predation by a broad range of natural enemies, involving predators, parasitoids, and pathogens. They often collectively termed aphidophaga. Because of their importance in biological control, the taxonomy and biology of aphid natural enemies have been the subject of numerous studies, books, and reviews. Generalist predators, including ladybirds (Coccinellidae), hoverflies (Syrphidae), lacewings (Chrysopidae) and aphidophagous midges (Itonididae) are well-known aphid natural enemies (Rotheray, 1989; Leroy *et al*., 2008). It is stated that they can have a significant impact in the suppression of aphid population growth and abundance (Chambers *et al*., 1986; Lucas *et al*., 1997; Lang, 2003; Lee *et al*., 2004).

Hoverflies (Diptera: Syrphidae) have an almost world-wide distribution and play an important role in insect communities. About 6000 species of which have been described (Vockeroth *et al*., 1987). The larvae of about 33% of these species, classified in the subfamily Syrphinae, are homopteran (usually aphids) predators (Rotheray, 1989; Gilbert, 1993). *Episyrphus balteatus* De Geer (Diptera: Syrphidae) is the most abundant species in central Europe (Tenhumberg & Poehling 1991; Colignon *et al*., 2001). It is one of the most efficient aphid-specific predators in natural agroecosystems, especially with respect to cereal aphids (Dean, 1982; Ankersmit *et al*., 1986; Chambers & Adams, 1986; Poehling, 1988; Entwistle & Dixon, 1989; Tenhumberg & Poehling 1995), *M. persicae* in tobacco field (Kalshoven, 1981) and *Brevicoryne brassicae* L. on brassica plants (Pollard, 1971). *E. balteatus* adults are flower visitors, while syrphid larvae are valuable aphid predators. The larvae of this syrphid species are predators on more than 100 species of aphids worldwide (Sadeghi & Gilbert 2000 a,b).

The predominance of hoverfly *E. balteatus* in natural agroecosystems as well as in natural habitats and its efficacy and potential as biological control agent for aphids may among others things, result from:

(i) Oviposition behaviour of females that oviposit always close to aphids colonies (Chandler, 1968 a,b,c; Scholz & Poehling 2000), and, thereby provide an immediate food source for the emerging larvae.

(**ii**) Its high reproductive rates (between 2000-4500 eggs per female) and voracity (in average 250 to 500 aphids per single syrphid larva) that allow it to efficiently exploit short-lived aphid colonies (Ankersmit *et al*., 1986; Chambers, 1988; Branquart & Hemptinne, 2000).

(iii) Its high mobility enables it to distribute eggs over large areas (Schneider, 1948; Chambers, 1988, 1991), and to locate aphid colonies earlier in the season than other aphidophaga (Hagen & van den Bosch, 1968; Horn, 1981; Dixon, 2000).

(**iv**) It is selective in choice of its oviposition site with high quality and is likely to exploit the encounters patches according to their relative value (Kan, 1988a,b).

(**v**) The short developmental time of larvae compare to other aphidophaga such as *Coccinella septempunctata* Linnaeus and *Chrysoperla carnea* Stephens, enables them to develop rapidly and to reduce in minimum the period of exposition of sensible new hatched larvae to risk of predations (Hindayana *et al*., 2001).

Apart from being an important group of naturally occurring aphid predators in field crops, aphidophagous hoverflies can be used efficiently like any other natural enemy for biological control (Kreß, 1996; Schneller, 1997). Recently, hoverfly *E. balteatus* has been successfully used in the biological control programs for aphid control (Copping, 2004; Pineda & Marcos-

García, 2008). There have been few attempts to demonstrate the potential *E. balteatus* in classical or augmentation biological control programmes. For example, in Switzerland, releases of syrphid larvae, together with ladybird larvae against the rosy apple aphid *Dysaphis plantagine* on apple seedlings reduced aphid densities to 5% of those on untreated control seedlings (Wyss *et al*., 1999b). In China, releases of *E. balteatus* larvae in vegetable fields at a ratio of 1:180 aphids reduced aphid populations by over 90% in 3 days (Yang *et al*., 2002). In Europe, *E. balteatus* was also considered for biological control of aphids on rose plants and sweet pepper in greenhouses (Kreß, 1996, Pineda & Marcos-García, 2006).

In addition to the importance of aphidophagous hoverflies in biological control of aphids, their foraging and oviposition behaviours are confronted to a diversity of environmental situations in which they should adopt different behavioural strategies. By their actions, they can influence the size, the structure and the population dynamics of their aphid prey and of other predators/parasitoids present in the same guild as well as on the overall community. Thus when hoverfly females attempt to forage, they must decide where to feed or oviposit for potential prey or hosts, what type of prey or hosts to accept and, when to move to a new habitat. The outcome of these decisions can greatly influence their fitness because neonate syrphid larvae have limited dispersal abilities in their search for food. Optimal oviposition theory predicts that *E. balteatus* females should lay eggs in aphid patches that are the most suitable for offspring development with high nutritional value and/or low risks of predation and competition pressure. It is stated that the choice of the oviposition site by aphidophagous hoverflies depends on several factors including host plant, aphid species, prey availability, semiochemicals emitted from aphids or their associated with host plants, the presence of intra- or interspecific competitors and female's age. On the basis of literature studies, little information is available about the behavioural responses of hoverfly *E. balteatus* females to these factors. The overall objective of this PhD thesis was to complete our knowledge of predatory hoverfly behaviour in response to these factors, and to understand the mechanisms of decision-making by syrphid females during their egg-laying behaviour, a crucial point before their effective use in biological control strategies of aphid populations.

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Chapter 2

Review of the Literature

The importance of aphidophagous hoverflies and their natural history, biology and ecology have been well documented. Previous reviews have largely discussed information on prey-predatory hoverflies interactions, and their degree of specialisation. However, the foraging and oviposition behaviour of predatory hoverflies have received little attention. In this chapter we wanted to summarise the available information about the searching and oviposition behaviour of aphidophagous hoverflies, and the behavioural mechanisms of decision-making by syrphid female during their egg-laying behaviour, taking into account that many factors involving in choice of oviposition site by hoverfly females such as: habitat, host plant, aphid species, prey availability, semiochemicals, presence of intra- or interspecific competitors and female age.

Article 1

Searching and oviposition behaviour of female aphidophagous hoverflies (Diptera: Syrphidae): a review

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Abstract – Aphidophagous hoverflies forage according two different host-finding mechanisms: they forage for suitable food sources (for their energy-expensive hovering flight, and for protein to mature their reproductive system), and for suitable oviposition sites. Syrphids are highly mobile, enabling them to lay eggs over large areas, and to locate aphid colonies earlier in the season than other aphidophaga. The result is that most syrphid eggs tend to be laid close to aphid colonies. The choice of oviposition sites may be crucial for offspring performance because the neonate larvae have limited dispersal ability. Selection of aphid patches should therefore reflect nutritional value, risk of predation and competition pressure. Several factors are known to affect the choice of oviposition site: host plant, aphid species, aphid availability, semiochemicals, the presence of intra- or interspecific competitors and female age. We review here the available information on these factors in order to understand the mechanisms of decision-making by syrphid females during their egg-laying behaviour, a crucial aspect of their effective use in strategies of the biological control of aphids.

Key words: Aphidophagous hoverflies, foraging and oviposition behaviour, Syrphidae, host choice, prey patch quality, semiochemicals, *Episyrphus balteatus*.

Résumé – Les syrphes aphidiphages utilisent deux mécanismes différents au cours de leur recherche et comportement de ponte, un mécanisme orienté visant à trouver une source de nourriture qui assure à la femelle d'avoir l'énergie nécessaire pour sa mobilité et la maturation de ses organes reproducteurs, et l'autre visant à trouver un site de ponte propice. Les femelles de syrphes prédateurs ont une forte mobilité qui leur permet de distribuer les œufs sur de larges territoires, et de localiser les colonies de pucerons plus tôt dans la saison plus que les autres prédateurs aphidiphages. Le résultat net est que la plupart des œufs de syrphe ont tendance à être déposés à proximité de colonies de pucerons. Cependant, le choix du site d'oviposition par les femelles peut être crucial pour la survie larvaire parce que les larves de syrphe ne peuvent pas se déplacer sur de longues distances pour la recherche de nourriture. C'est pourquoi les femelles gravides devraient montrer une préférence pour les sites présentant une grande valeur nutritive et un faible risque de prédation et de compétition. Pour les syrphes aphidiphages, plusieurs facteurs influencent le choix du site de ponte parmi lesquels : la plante hôte, l'espèce de pucerons, la taille de la colonie de pucerons, les substances sémiochimiques émises par les pucerons et leurs associations avec leurs plantes hôtes, la présence de compétiteurs intra- ou interspécifiques et l'âge de la femelle. Dans cette revue bibliographique, nous recensons les informations disponibles sur ces facteurs afin de comprendre les mécanismes de décision prise par les femelles de syrphes prédateurs au cours du comportement de ponte, ce qui constitue une étape importante avant d'utiliser ces prédateurs dans la lutte biologique contre les pucerons.

Mots clés : Syrphes aphidiphages, comportement de recherche et de ponte, Syrphidae, choix de l'hôte, qualité de la proie, sémiochimiques, *Episyrphus balteatus.*

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Introduction

Although a good number of insects do not feed as adults, for the majority, most of their adult life is dedicated to actions related both to the acquisition of food and to reproduction, and crucial decisions must be taken concerning these two activities. Oviposition behaviour is a vital component of many aspects of insect biology (e.g. population dynamics, life history and biological control of insect pests). One major aspect of oviposition behavior is host selection. Offspring are often obliged to feed on the host chosen by females during their egg-laying behaviour. Optimality theory as applied to oviposition predicts that female choice should reflect a preference for oviposition sites with high expected fitness for their offspring, usually in the form of high nutritional value, low risk of predation and competition pressure (Mangel, 1987), good growth, survival, and future reproductive potential, etc.: eggs deposited in unsuitable hosts are likely to die or result in inferior adults (Nufio & Papaj, 2004; Singer et al., 2004).This relationship is especially important for insect species where neonate offspring are relatively sessile and have limited mobility to forage (Thompson, 1988; Peckarsky et al., 2000).

During foraging and oviposition behaviour, entomophagous insects are confronted with a diversity of environmental situations in which they may adopt different behavioural strategies. By their actions, they can influence the structure and population dynamic of their hosts or prey and of other predators/parasitoids present in the same guild and of the overall community (Jervis & Kidd, 1996). They can also mediate interactions between insect herbivores and their host plants, thereby constituting a selective factor on herbivore host plant preference (Price et al., 1980). Thus when an individual attempt to forage, it must decide where to feed or oviposit for potential prey or hosts, what type of prey or hosts to accept and, when to move to a new habitat (Barnard, 1983). The outcome of these decisions can greatly influence the survival and fitness of predators and parasitoids. In trying to understand what influences the decision processes of foraging insects, ecologists have increasingly turned to optimal foraging theory (Stephens & Krebs, 1986; Scheirs & De Bruyn, 2002).

How do these ideas work out in the relationship between predatory hoverflies and their aphid prey? Aphids are considered to be major pests in most agricultural ecosystems (van Emden & Harrington, 2007). They have distinctive characteristics that make them highly suitable in some ways and highly challenging in others as prey for insect predators. On the one hand, aphids have small and soft bodies, and their higher growth and development rates enable them to occur at high densities. On the other, aphid colonies are ephemeral and unpredictable over both space and times, requiring special adaptations to be able take advantage of them. Aphidophagous predators such as predatory hoverflies therefore need appropriate tactics and strategies to locate aphid infestation quickly, and to exploit the opportunities and overcome the challenges posed by this particular group of prey.

Aphidophagous hoverflies have long been recognized as important aphid natural enemies (Chambers, 1988). The larvae of species such as *Episyrphus balteatus* DeGeer are predators on more than 100 species of aphids worldwide (Sadeghi & Gilbert, 2000b). Because of their high reproductive rates and voracities (Chambers & Adams, 1986; Poehling, 1988; Gilbert, 1993; Tenhumberg & Poehling, 1995) and suitable oviposition behaviour (Kan & Sasakawa, 1986; Kan, 1988a; Sadeghi & Gilbert, 2000a), they can have a significant impact in the suppression of aphid population growth and abundance, but good evidence is rare.

The ability to detect aphids and oviposit close to aphid colonies plays a major role in the effectiveness of predatory hoverflies. A high rate of prey search is considered to be one of the most desirable attributes of biological control agents (Jervis & Kidd, 1996; Murdoch & Briggs, 1996). The relatively sessile nature of neonate syrphid larvae does not allow them to exploit aphid prey on different host plants (Chandler, 1969). Moreover, they do not perceive aphids before contact or only at very short distance (Bargen et al., 1998). The female's ability to find and oviposit within the future foraging range of its progeny is therefore a critical determinant of potential biocontrol performance. The reason for the poor progress in developing a foraging theory for insect predators is that most studies have concentrated on the most voracious stage, the larva, rather than the adult. Thus for a complete understanding of insect predator-prey dynamics, it is necessary to determine the behavior that maximises predator fitness, and this involves studying the foraging and oviposition behavior of female predators (Ferran & Dixon, 1993). Gilbert (1993) has described the importance of predatory hoverflies and their natural history, biology and ecology. Recent reviews have largely discussed information on prey-predatory hoverflies interactions (Rojo et al., 2003), and the degree of specialization of aphidophagous syrphids (Gilbert, 2005). However, there is no review on the searching and oviposition behavior of aphidophagous hoverflies. Here, I summarize available information about the foraging and oviposition behaviour of aphidophagous hoverflies, and the behavioral mechanisms of decision-making by syrphid female during their egg-laying behavior.

Factors influencing searching and oviposition behavior of aphidophagous hoverflies

The choice of habitat, host plant, aphid species, aphid colony size, visual and chemical stimuli, oviposition site, must all be considered during searching and egg-laying behavior of syrphid predators. This review will discuss largely the main factors influencing searching and ovipostion behavior of predatory hoverflies.

Habitat

Searching for resources is one of the most important activities of gravid female insects (Bell, 1990). According to classical foraging theories, foragers maximize energetic gains by selectively exploiting patches rich in resources and by minimizing foraging time in poor patches (Stephens & Krebs, 1986). Adults predatory syrphids are frequent flower visitors since they feed only on nectar and pollen: nectar serves principally as a source of energy to sustain their strong flight and to extend longevity, while pollen allows maturation of the reproductive system in both sexes (Schneider, 1948, 1969; Gilbert, 1981; Chambers, 1988). Additionally, flowers can also provide optical cues such as size, color, shape and scent influencing the searching behavior of syrphid predators (Kan, 1988a,b; Haslett, 1989; Lunau, 1993; Sutherland et al., 1999). Floral cues are important signals in helping foraging hoverflies to find and select a floral feeding site, and the foraging activity of hoverflies in crops can be enhanced by a continuous supply of flowers with easily available pollen sources such as Asteraceae and Umbelliferae in field margins (Ruppert & Molthan, 1991; Colley & Luna, 2000, Morris & Li, 2000). For example, cereal fields are usually characterized by shortage of food for flower visitors, and alternative agricultural practices that favor wild flowers (i.e. set-aside, herbicide-free buffer zones, conservation strips) may lead to improved attraction of adult hoverflies (van Emden, 2003). Ambrosino (2006) showed that the presence of floral resources in Oregon broccoli fields enhanced the predatory potential of hoverflies on aphids. This seem to indicate that young syrphid females probably focus on flower foraging during the first week after emergence before switching to searching for aphids and oviposition sites. Thereafter, they will travel between floral and aphid patches to maintain egg production (van Rijn et al., 2006).

The important second stage of the foraging behavior of syrphid females is to locate a suitable oviposition site. Hoverfly females are known to exhibit high mobility, enabling them to distribute eggs over large areas (Schneider, 1948; Chambers, 1988), and to locate aphid colonies earlier in the season than other aphidophaga (Hagen & van den Bosch, 1968; Horn, 1981; Dixon 2000). The key questions facing searching hoverfly females can be summed up simply: when and where in the course of their search should they oviposit? and what are the cues and behavioral mechanisms involved in choosing their oviposition site?

An elegantly simple model of hoverfly oviposition behavior emerged during the 1960s. Female aphidophagous hoverflies are highly mobile and their ability to select a potentially successful oviposition site therefore merely depends upon the availability of aphid-infested plants (Dixon, 1959; Chandler 1968a,b; Schneider, 1969). This model has served since as the standard general explanation for the degree of discrimination exhibited by syrphid females in selecting an oviposition site, and the various stimuli which induce oviposition responses in particular hoverfly species. Laboratory and field experiments and observations over the past several decades have generally supported this model. The net result of searching and oviposition behavior is that syrphid eggs tend to be laid close to aphid colonies (Chandler, 1968a,b; Chamber, 1988; Dixon, 2000; Scholz & Poehling, 2000; Ambrosino et al., 2007), enabling the emerging young larvae to locate the food sources immediately. Syrphid predators may conduct an intensified local search after locating aphids. Field and laboratory observations have showed that a female approaches an infested plant in a straight line, and then hovers, moving slowly around plants until it reaches a position opposite and close to an aphid colony, where it hovers a short time before alighting with the ovipositor extended. Finally, the ovipositor is bent ventrally and drawn over the substrate and an egg is laid (Dixon, 1959; Schneider, 1969; Scholz & Poehling, 2000; Sutherland et al., 2001; Almohamad et al., 2008c).

Oviposition sites are very variable, and are related both to the number and location of eggs deposited. Syrphid eggs are often laid singly, either close to or within aphid colonies, although some species lay eggs in batches distant from the colony or even on uninfested plants (Chambers, 1988). In the latter case, young larvae may survive by cannibalizing conspecific eggs.

In the field, aphids of different species have been found with syrphid eggs actually attached to them, which demonstrates how close eggs can be laid to aphids (Dixon, 1959). In certain melanostomine and all *Platycheirus* species except *Platycheirus scutatus* (Meigen), eggs are equally often deposited on plants without as on those with aphids (Chandler, 1968a; Gilbert, 1986), and eggs of the latter species are laid in batches of two to four, instead of singly (Gilbert, 1986). The net effect is that these species exploit small aphid colonies that do not attract species such as *Syrphus ribesii* L. and *Eupeodes* (*Metasyrphus*) *corollae* Fabr. : the first larva to hatch can cannibalize the others and then search for aphids, and females lay in advance of aphid attack. In species such as *Pipizella varipes* Meig., females select as oviposition sites the base of stems of *Pastinaca sativa* L. (Apiaceae) plants, the roots of which are infested with aphids just below soil level, although the aphids are not visible to ovipositing flies (Dixon, 1959). Choice of oviposition site varies markedly according to hoverfly species, even in similar conditions: female *Eupeodes luniger* Meigen, laid over 50% of their eggs touching aphids (*Brevicoryne brassicae* L.) on Brussels sprouts (*Brassicae oleracea gemnifera* L.) and less than 1% on uninfested plants, whereas female *Platycheirus manicatus* Meig. laid less than 5% touching aphids and over 50% on uninfested plants (Chandler, 1968c). *Epistrophella emarginata* (Say) oviposits on the petioles of leaves and *Syrphus knabi* Shannon usually on the upper surface of the lamina (Curran, 1925). Laboratory observations have also demonstrated that *Episyrphus balteatus* females have a distinct preference of position to lay their eggs on *Vicia faba* L. plants: 91% were found on the bottom side of infested leaflets, 2% on the upper side, and 7% of eggs mainly on the top of plant (Scholz & Poehling, 2000).

Host plant

According to Cortesero et al. (2000), host-plant effects on the efficiency of insect natural enemies can occur in various ways, such as by mediating host/prey accessibility and availability, providing host/prey finding cues, influencing host/prey suitability and providing supplemental food resources.

Several studies have showed that host-plant factors play important roles in the selection of oviposition site by aphidophagous hoverflies (Dixon, 1959; Chandler, 1968b; Sanders, 1983a,b; Sadeghi & Gilbert, 2000a; Almohamad et al., 2007a). Most syrphid species are known to lay their eggs close to aphid-infested plants, whereas other species (i.e. *Melanostoma* spp. and *Platycheirus* species) tend to lay their eggs freely on uninfested plants. Thus, the existence of species that oviposit in the absence of aphids may be valuable in biological control, and provides a useful tool for the investigation of non-aphid oviposition stimuli. Some host-plant factors affecting oviposition were clearly showed in the study of Chandler (1968b): plant species, plant appearance and substrate of plant surface were all important. Species such as *Platycheirus* spp. preferred waxy over glossy varieties of brussel sprout (*Brassica oleracea* L.) (Brassicaceae) if they were uninfested, but this preference was much less marked if the plants were infested; *Eupeodes* spp. preferred glossy plants when both types were uninfested, but not if the plants were infested; *Melanostoma* spp. preferred waxy plants irrespective of the presence or absence of aphids; *Sphaerophoria* spp. responded more like *Platycheirus* than *Eupeodes*. Other species could seemingly discriminate and select plants on the basis of their appearance, although different contact stimuli may also have mediated oviposition. The nature of the plant surface substrate affects the number of eggs laid per patch (batch size) in species of *Melanostoma* and *Platycheirus* (Chandler, 1968b).

It has been also suggested that there is a balance between aphid and host-plant factors governing syrphid oviposition. If the aphid stimulus is reduced, by scarcity or absence, or if the female is old, host-plant factors become more important (Dixon, 1959; Chandler, 1967; Chandler, 1968b; Schneider, 1969; Sadeghi & Gilbert, 2000c). Evidence for this is discussed in several studies. For example, *Platycheirus manicatas* females oviposit selectively on healthy brussel sprout or bean plants adjoining those heavily infested with aphids (cabbage aphid *Brevicoryne brassicae* L.,and bean aphid *Aphis fabae* Scopoli, respectively) (Chandler, 1968b). Oviposition responses to host plants with low aphid infestations may be especially good at keeping aphids at low densities.

Plant chemistry (allelochemicals or secondary plant metabolites) can also affect the foraging and oviposition behavior. Studies in the literature have largely focused on host-plant chemistry effects on the suitability of aphid prey for overall performance and subsequent fecundity, but few studies have compared the performance of syrphid larvae feeding on one aphid species but from different host plants (Schmutterer, 1972; Rüzicka, 1975; Sadeghi & Gilbert, 2000b; Hindayana, 2001; Vanhaelen et al., 2001, 2002, Almohamad et al., 2007a). Oviposition responses to different host plants associated with one aphid species have received little attention, with only two studies. Vanhaelen et al. (2001) demonstrated that *Episyrphus balteatus* females significantly prefer to oviposit on white mustard plants (*Sinapis alba* L. containing high glucosinolate (GLS) levels) rather than on oilseed rape plants (*Brassica napus* L. containing low GLS levels), both of which were infested with the same aphid species (*Myzus persicae* Sulzer). GLS compounds are well known allelochemicals of the Brassicaceae, with a strong influence on both the phytophages and entomophages of the community (Francis et al., 2001). Almohamad et al. (2007a) recently showed that potato plants *Solanum tuberosum* L. were preferred by ovipositing *Episyrphus balteatus* females over Black Nightshade plants *Solanum nigrum* L. infested with the same aphid species (*M.*

persicae). The importance of volatile compounds (e.g. E-(β)-farnesene: EβF) emitted from these aphid-host plant combinations may explain these oviposition preferences. Further investigations are needed to understand better oviposition activity in relation to plant allelochemicals, and the consequent effect of this on offspring performance.

Some physical plant characteristics (e.g. presence of trichomes) have also been shown to influence the acceptance of aphid/host plant as oviposition site. Field observations have demonstrated that that nettle (*Urtica dioica* L.) infested with *Microlophium carnosum* Buckton was poorly accepted by ovipositing *Episyrphus balteatus* females (Sadeghi & Gilbert, 2000a), but it is unclear whether physical aspects of the plant were influential. A variety of factors affect the evolved rank hierarchy of suitability: the host plant as a habitat for larvae; the intrinsic suitability of the aphid as food (which may vary with host plant: Hodek, 1993). Thus the survival of *Episyrphus balteatus* larvae on nettles in nature may be low because of the physical effects of this host plant itself on the larvae, which must be able to move on its surface. Nettle aphids are also known to be especially adept at avoiding capture (Sadeghi & Gilbert, 2000a). All these reasons could underlie the fact that the combination *M. carnosum/ U. dioica* was the least preferred aphid by *Episyrphus balteatus*.

Other host plant factors (e.g. floral characters, color) are found to have important impact on searching and oviposition behavior. Several researches conducted in both North America and Europe indicate that aphidophagous species such as *Episyrphus balteatus* exhibit considerable positive and negative selectivity for native flowering species (Cowgill et al., 1993; Branquart & Hemptinne, 2000; Fitzgerald & Solomon, 2004). Branquart & Hemptinne (2000) showed that adults have a strong flower preference for pollen and nectar produced by native plants with large inflorescences and flat corollas (i.e. Apiaceae, Asteraceae, Ranunculaceae and Rosaceae). These authors also suggested that several polyphagous species such as *Episyrphus balteatus*, *Melanostoma mellinum* L., *Eupeodes corollae*, *Sphaerophoria scripta* L. and *Platycheirus* spp. can access pollen and nectar in flowers with small tubular corollas, an important asset for colonizing open and ephemeral habitats. Indeed, flowers are considered to have important effects on distribution and oviposition in neighbouring aphid–infested plants. For example, work from New Zealand has shown that syrphids move into adjacent crops (brassica crops) from rich floral patches (of *Phacelia tanacetifolia* Benth.: Hydrophyllaceae), where they oviposit, and the subsequent larvae can cause a decrease in aphid populations (White et al., 1995). MacLeod (1999) also demonstrated that species such as *Episyrphus balteatus* were significantly more abundant on arable field margins with rich floral resources than those with no additional floral resources; and yet another study showed that the presence of floral resources in Oregon broccoli fields enhanced predatory potential (Ambrosino, 2006). As result, managing hedgerows and field margins to create florally rich habitats to attract and retain syrphids is an option farmers can consider to encourage them into fields as part of a system of integrated pest management.

In conclusion of this section, host-plant factors are likely to be very important in the foraging and oviposition behavior of aphidophagous hoverflies.

Aphid species

Aphid species differ in their profitability and suitability for insect predators. Hodek (1993) has distinguished several types of prey for aphidophagous predators. One such division was suitable vs. unsuitable prey. Suitable prey could function either as essential, enabling larval development and egg production, or as alternative, enabling just survival or accumulation of energetic reserves for overwintering (Hodek & Honek, 1996). Unsuitable prey, which can include toxic species, can be either rejected or accepted. This classification arose from finding that several aphid species were accepted but unsuitable, (i.e. they were inadequate for larval development or oviposition). Michaud (2005) also stressed that the suitability of prey sometimes differed for larval development and adult reproduction.

Aphidophagous hoverflies are likely to encounter diverse aphid species when foraging for an oviposition site. Selection among aphid species should reflect a preference for high expected offspring performance (Scheirs & De Bruyn, 2002). Ovipositing females do appear to discriminate among different food types, and appear to have a rank order hierarchy of preference for aphid prey species or aphid-host plant combinations. Females become less selective with increasing age, but the rank hierarchy is preserved (Sadeghi & Gilbert, 2000a,b). The hierarchy-threshold model (Courtney et al., 1989; Sadeghi & Gilbert, 2000a,b) can be applied to a gravid female syrphid searching among a set of possible prey (A to E) (Figure 1). In this model, females possess an intrinsic evolved degree of preference for each food type, producing a rank order of preference among prey that does not change throughout an individual’s lifetime; individuals accepting a low-ranking food type will also accept all higher ranking types; and actual acceptance of an encountered type depends on whether the stimulus of that food type exceeds the current motivational threshold (which can vary with factors such as age or egg-load). The model is particularly useful because it synthesizes two disparate strands of adaptive explanations of specialization (Berenbaum, 1990), one involving slow processes of evolutionary change based usually on various sorts of trade-off and coevolution, and the other invoking optimal foraging and concentrating on the behavioral flexibility of the individual in response to variation in ecological conditions, acting via "rules of thumb".

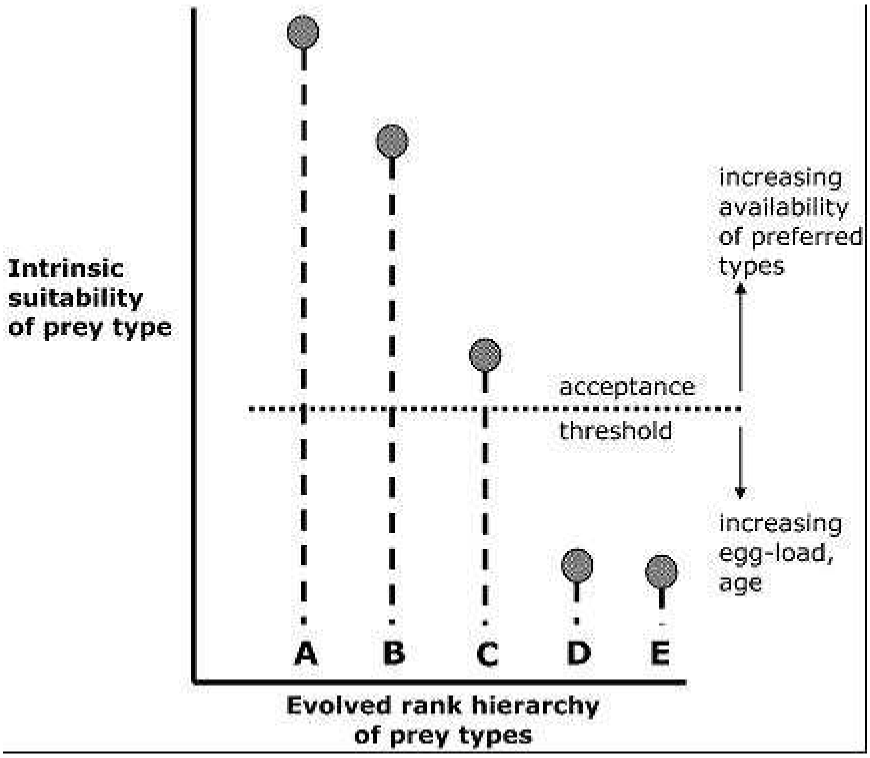

Figure 1. Hierarchy-threshold model of host choice (Courtney et al., 1989) applied to a gravid female syrphid searching among a set of possible prey (A to E).

Field observations have showed that gravid females of generalists such as *Episyrphus balteatus* and *Syrphus ribesii* exhibit significant preferences in the distribution of their eggs among various aphids in natural habitats (Budenberg & Powell, 1992; Sadeghi & Gilbert, 2000a; Almohamad et al., 2007a). Even greater selectivity may reasonably be predicted in specialists such as *Xanthandrus* (Lyon, 1968) and *Platycheirus fulviventris* Macquart (Rotheray & Dobson, 1987). Because most species are oligophagous (Gilbert & Owen, 1990), predatory hoverflies are clearly like insect herbivores in that most species are relatively specialized (Schoonhoven et al., 1998). However, it has been also reported that there are varying degrees of specialization among individuals within the populations of at least one generalist, *Episyrphus balteatus* (Sadeghi & Gilbert, 1999). Some individual females differ from others in their preferences, and at the individual level there appeared to be life-history trade-offs in performance with these preferences. Thus part of the female population of *Episyrphus balteatus* seems to be specialized to particular aphids as prey; the rest of the population may also be specialized, but to aphid species not tested in the study, or may consist of truly generalized individual females.

There are rather few studies that investigate oviposition preferences in response to different aphid species. A good example of such a study is that of Budenberg et al., (1992), who found that *Episyrphus balteatus* females lay their eggs in response to some aphid species such as rose-grain aphid *Metopolophium dirhodum* Walker and pea aphid *Acyrthosiphon pisum* Harris, but not to others such as the nettle aphid *M. carnosum*. This preference is expressed in response to honeydew alone. In another study, *A. pisum* and *Macrosiphum rosae* L. were clearly more preferred hosts of *Episyrphus balteatus* and *Syrphus ribesii* females, and *M. carnosum* and *Aphis ruborum* (blackberry aphid) were the least preferred (Sadeghi & Gilbert, 2000a), results consistent with the field distribution of larvae (Table 1). In the study of Almohamad et al., (2007a), the foraging and oviposition behavior of *Episyrphus balteatus* females was evaluated in response to different aphid species (*A. pisum*, *A. fabae* and *Megoura viciae* Buckton) infesting one host plant (*V. fabae*). *A. pisum* and *M. viciae* were equally attractive, whereas *A. fabae* was less attractive.

In addition to their ability to reduce aphid abundance, aphid predators can also cause changes in prey characteristics by inducing defensive responses that help prey avoid being consumed; these often come at a cost to some other aspect of prey biology. Aphids possess a range of defenses against predators, including morphological, social, chemical, and behavioral defenses (Losey & Denno, 1998). These behavioral responses may affect suitability for syrphid females. A beautiful work on aphid defense against syrphid predators is a study by Shibao (1998). He clearly demonstrated that gravid female *Eupeodes confrater* (Wiedemann) adjusted their oviposition behavior in response to soldier density in its prey, the bamboo aphid *Pseudoregma bambucicola* (Takahashi). This aphid has huge colonies and a soldier caste for colony defense who pierce the eggs and neonate larvae of aphid predators. A gravid female of *Eupeodes confrater* circles the colony carefully: if she finds soldiers present, then she lays a batch of eggs on a spider’s web nearby, up to 1 m away. The first larva to emerge cannibalizes the rest of the batch to provide the energy to crawl to the colony: having a meal or two before meeting a soldier will make all the difference between surviving and succumbing. If the gravid female does not encounter any soldiers in her search, then she lays single eggs in the colony, as is normal for most aphidophagous syrphids. Another species, *Eupeodes hakkiensis***,** has adapted to dealing with the aggressive soldier instars of their *Ceratovacuna* aphid prey by evolving a hard impenetrable eggshell, and larval behavior that leads them to forage only at the edge of the colony, moving away when not feeding (Mizuno et al., 1997).

Table 1. The most- and least-preferred aphid prey of *Episyrphus balteatus* and *Syrphus ribesii*, as assessed from field distribution (data from Sadeghi, 2000 and P. Láska, unpubl.).

Hoverfly			
Episyrphus balteatus		***Syrphus ribesii***	
Most-preferred aphid prey	**Least-preferred aphid prey**	**Most-preferred aphid prey**	**Least-preferred aphid prey**
Aphis grossulariae on willow-herb	*Phyllaphis* on beech	*Drepanosiphum* on sycamore	*Phyllaphis* on beech
Cavariella on hogweed	*Aphis ruborum* on blackberry	*Cavariella* on hogweed	*Aphis grossulariae* on willow-herb
Macrosiphum rosae on rose	*Microlophium carnosum* on nettle	*Microlophium carnosum* on nettle	*Aphis ruborum* on blackberry
Schizoneura on elm	*Aphis pomi* on apple	*Macrosiphum rosae* on rose	*Aphis pomi* on apple
Brevicoryne brassicae on cabbage	*Aphis fabae* on bean	*Aphis sambuci* on elder	*Uroleucon* on cichory
Aphis fabae on thistle	*Aphis sambuci* on elder	*Myzus on Wild cherry*	*Aphis fabae* on spindle
Aphis fabae on spindle	*Myzus cerasi* on wild cherry		
Hyalopterus on reed	*Uroleucon* on cichory		
Rhopalosiphum on bird-cherry	*Drepanosiphum* on sycamore		

Aphid size may also be an important characteristic in determining oviposition choice. Kan (1988a,b) noted that aphid size is critical for the newly eclosed first-instar larva, and part of the reason for ovipositing in young colonies may be to make available small and tender aphids for the first few meals of the first instar. However, small size is not good in the longer term, over the entire developmental period: the higher mortality and longer development time for *Episyrphus balteatus* larvae feeding on apple aphid (*Aphis pomi*) may be related to the small size of individuals of this aphid, which imposes extra capture costs on older larvae and makes it a least-preferred aphid by hoverfly females (Table 1). Further consequence is that normal colony size and density of aphid species may be one reason why blackberry aphids (which often occur at very low densities) are low in the oviposition preference hierarchy (Table 1). Newly emerged larvae must have enough food to develop successfully, and periods of food deprivation during the larval stage can result in dwarfed adults (Rüzicka & Gonzales Cairo, 1976) with lowered fecundity or even sterility (Cornelius & Barlow, 1980). Michaud & Belliure (2001) showed that *Pseudodorus clavatus* F. could hamper the population growth of the brown citrus aphid by decreasing production of the winged form.

Prey availability

A predator that responds numerically to increasing aphid numbers and oviposits accordingly is thought to be ideal for suppressing pest populations before they reach damaging levels (Murdoch & Briggs, 1996). Predatory hoverfly larvae exploit temporary aphid colonies as food resources in crops and on a wide range of herbaceous plants (Salveter, 1996). Aphid colonies are ephemeral, patchily distributed resources (Dixon, 1959; Kan, 1988a,b), suddenly disappearing due to predation, parasitism, fungal epizootics, declining host-plant quality, changes in weather, or dispersal. Syrphid larvae therefore face a potentially unstable food supply, and hence it may be important to be able to locate aphid infestations quickly. According to Horn (1981), adult syrphids appear to be especially adept at locating aphid colonies because of their strong flight and ability to hover and inspect foliage for aphids. For example, *Episyrphus balteatus* females are able to find even small and isolated aphid colonies (Itô & Iwao, 1977). High levels of oviposition can therefore occur relatively early, and large numbers of larvae can hatch before aphid populations have attained rapid growth rates (Tenhumberg & Poehling, 1992; Ambrosino et al., 2007).

Several studies have demonstrated that oviposition varies with the size of aphid infestations (Dixon, 1959; Chandler, 1968b; Kan, 1988b; Bargen et al., 1998; Scholz & Poehling, 2000; Belliure & Michaud, 2001; Sutherland et al., 2001; Almohamad et al., 2006; Ambrosino et al., 2007). This behavior has been attributed to a ‘buy futures’ tactic of oviposition whereby foraging females are selecting aphid colony sizes based on their future potential rather than their immediate value (Kan, 1988b). Different species have indeed different optimum aphid population sizes for oviposition. A very good example is the study of Chandler (1968b) on the relation between aphid infestation and syrphid oviposition in field. He found that *Platycheirus manicatus* preferred about 100 aphids per plant, *Platycheirus scutatus* about 1000, *S. ribesii* about 2000, whereas *Sphaerophoria scripta* had no obvious preference. Other species such as *Eupeodes luniger* preferred small numbers of large aggregates to a large number of smaller ones, whereas *Episyrphus balteatus* preferred the opposite. Other studies have also demonstrated that *Episyrphus balteatus* prefers smaller aphid colonies, or aphid colonies with a high proportion of early aphid instars (Kan & Sasakawa, 1986; Kan, 1988a,b; Hemptinne et al., 1993). In the study of Ambrosino et al. (2007), the numbers of eggs were very low on broccoli plants with fewer than 50 aphids, and none were seen on leaves that had more than 400 aphids. Thus the tendency of the different species to select aphid populations of different sizes and distribute their eggs accordingly could reflect adaptations that reduce interspecific competition.

There no evidence of a peak in hoverfly oviposition at higher aphid numbers at the plant level. Other factors may influence this, for example the quantity of volatile compounds emitted from aphids (such as EβF: Almohamad *et al*., 2008b), and their liquid secretions (such as honeydew: Budenberg & Powell, 1992; Sutherland et al., 2001). EβF has an attractive effect on *Episyrphus balteatus* females and acts as an oviposition stimulant (Almohamad et al., 2008c), and honeydew acts as a contact kairomone and oviposition stimulant (Budenberg & Powell, 1992; Sutherland et al., 2001). Sutherland et al. (2001) also reported that females demonstrated more gustatory and oviposition responses to honeydew-treated areas. With aphid alarm pheromone (EβF), honeydew might also provide females with information about aphid colony size.

Syrphid eggs and larvae are also more exposed to cannibalism and/or the risk of starvation if the aphid colony on which they are feeding disappears before they complete their development. This could happen when too many eggs are laid in the colony or too late in the development of the colony, i.e., when the aphids are preparing to disperse. Evaluation of the aphid colony by females, and directed prey location by larvae, would therefore be favored, resulting in lower larval mortality and subsequently higher reproductive success (Kindlmann & Dixon, 1993; Almohamad et al., 2007b). Thus females manifest evolved behavioral mechanisms in response to aphid colony size that enable them to forage for an oviposition site that will support the development of their offspring.

Semiochemicals

Choice of oviposition site is described as a process of recognition, often depending on the development phase of the searching insect and on the cues available (Schoonhoven et al., 1998). Host choice involves a number of actions, from initial perception of the host, through testing stages by different sensory systems, until the final decision of rejection, or acceptance: i.e. laying eggs (Bernays, 1996), all of which may involve semiochemicals mediating these actions (Dicke, 1999; Ninkovic et al., 2001; Harmel et al., 2007; Verheggen et al., 2008). These chemical signals emitted from plants or aphid host plant can be considered a part of the indirect defense of plants against herbivores (Harmel et al., 2007). Studies in the literature have largely focused on the role of semiochemicals emitted by aphid prey or associations with their host plants on various aphid natural enemies, including ladybeetles, and parasitic hymenoptera (Du et al., 1998; Francis et al. 2004). Little information is available about the role of semiochemicals in searching behavior and acceptance of oviposition sites by predatory hoverflies (Laubertie et al., 2006; Verheggen et al., 2008; Almohamad et al., 2008a).

Field and laboratory experiments have showed that females are able to find even small and isolated aphid colonies (Chambers, 1991). Behavioral observations show that they do not approach aphid-infested plants directly, but slowly scan close to non-infested plants and non-infested parts of infested plants in search for aphids, and only remain stationary directly front of aphid-infested plants (Dixon, 1959; Scholz & Poehling, 2000). This suggests that foraging behavior is not simply a random search for prey, but is instead guided by specific volatiles or substrate-linked semiochemicals. Thus oviposition is almost certainly elicited by both olfactory and visual cues. Female *Eupeodes corollae* and *Episyrphus balteatus* respond positively to stimuli originating from aphid honeydew, and probably also to ones from aphid siphunculus secretion. Such stimuli may act both as long-distance kairomones and oviposition stimuli after the location of a plant with prey (Volk, 1964; Budenberg & Powell, 1992; Bargen et al., 1998; Shonouda et al., 1998; Sutherland et al., 2001). Additionally, female *Eupeodes corollae* respond to structural characters of plants, having a preference for vertical rather than horizontal surfaces and preferring darker to lighter strips (Sanders, 1983a; Chambers, 1988). *Episyrphus balteatus* females also respond to leaf color (Sutherland et al., 2001).

In aphidophagous hoverflies, it has been suggested that there are four stages in the location and acceptance of an oviposition site. During these stages, a range of different ovipositional cues (visual, auditory, olfactory and gustatory) are used (Table 2).

In the first stage of searching behavior, females use long-range optical cues, including the size, density and color of the stand of vegetation, to help them find suitable oviposition sites (Chandler, 1966; Sanders, 1982; Lunau, 1993; Sutherland et al., 1999; Laubertie et al., 2006). Short-range optical cues are then thought to operate in the second stage, which involves aphid-colony recognition (Dixon, 1959; Sanders, 1983a,b; Kan & Sasakawa, 1986; Sutherland et al., 2001). Several studies have shown that females oviposit in response to volatile compounds emitted from aphids and their liquid secretions such as honeydew (Dixon, 1959; Budenberg & Powell, 1992; Shonouda et al., 1998; Verheggen et al. 2008; Almohamad et al., 2008b).

Table 2. Role of semiochemical cues emitted from aphid and host plant in searching and egg-laying behavior of hoverfly females.

Sense involved	Influences	Reference
Visuals cues	1. Size of plant patch	Chandler, 1968a; Sanders, 1983a,b
	2. Density of plant patch	Chandler, 1966; Chandler, 1968c
	3. Colour of plants	Sanders, 1982 ; Sutherland et al., 1999 ; Laubertie et al., 2006
	4. Form of plant	Chandler 1968a; Sutherland et al., 1999
	5. Size and position of aphid colony	Chandler, 1968b; Itô & Iwao, 1977; Bargen et al., 1998; Scholz & Poehling, 2000; Sutherland et al., 2001; Almohamad et al., 2006
	6. Shape of aphids	Chandler, 1968b
	7. Movement of aphids	Chandler, 1968b; Itô yet al., 1977
Olfactory cues	8. Smell of plants	Sutherland et al., 1999; Verheggen et al., 2008
	9. Smell of aphids	Volk, 1964 ; Almohamad et al., 2008c; Verheggen et al., 2008
	10. Smell of aphid associated with plants	Volk, 1964; Harmel et al., 2007; Verheggen et al., 2008
Gustatory cues	11. Honeydew	Dixon,1959; Kan et al., 1986; Budenberg & Powell, 1992; Scholz & Poehling, 2000; Sutherland et al., 2001
Touch	12. Actual site for eggs	Dixon, 1959; Schneider, 1969

Response by females	Influences involved
Habitat selection	1, 2, 3
Plant selection	2, 3, 8
Aphid colony selection	5, 6, 7, 9, 11
Egg-site selection	5, 6, 7, 12

The third (penultimate) stage involves the processing of olfactory stimuli. There is an apparent dichotomy in behavioral responses to olfactory stimuli, identified by Chandler (1968c): (1) phytozetic species, such as *Melanostoma mellinum*, rely more on plant–derived stimuli than on aphid location; and (2) aphidozetic species, such as *Episyrphus balteatus*, use aphid-derived chemicals to locate their prey and subsequent oviposition sites. There are few published works on the role of chemical odors in hoverfly attraction, but a very good example is the study of Verheggen et al. (2008), who tested the olfactory responses of *Episyrphus* *balteatus* to several aphid and plant volatiles, including terpenoids (mono-and sesquiterpenes) and green leaf volatiles ((Z)-3-hexenol, (*E*)-2-hexenol, (*E*)-2-hexenal and hexanal). They found that monoterpenes induced significant responses, whereas sesquiterpenes were inactive, except for the aphid-alarm pheromone (*E*)-β-farnesene. Some chemical volatiles ((Z)-3-hexenol and EβF) caused orientation toward the host plant, and stimulated egg-laying, suggesting that oviposition site selection depends on the perception of odors released from aphids, plants, or aphids in association with particular host plants. Francis et al. (2005) showed that *Episyrphus balteatus* larvae are guided by olfactory cues from aphids to locate their aphid prey. Almohamad et al. (2008c) found that *Episyrphus balteatus* females respond positively to the odor of (E)-β-farnesene, but not to the odor of geranyl acetone.

In the final stage, gustatory stimuli (proboscis extention) are used in response to aphid liquid secretions such as honeydew, and they then exhibit an abdominal protraction or oviposition (Dixon, 1959; Budenberg & Powell, 1992). Honeydew is also known to serve as an important oviposition stimulus for *Episyrphus balteatus* females (Budenberg & Powell, 1992, Bargen et al., 1998; Sutherland et al., 2001).

Intraguild interactions (the presence of intra-and interspesific competitors)

In addition to their ability to reduce aphid populations effectively, aphidophagous hoverflies do not exist in isolation but generally are part of larger complexes within the aphidophage guild (Rosenheim et al., 1995; Hindayana et al., 2001; Lucas, 2005). Syrphids can act as intraguild (IG) - predators against other aphid predators (Hindayana et al., 2001; Fréchette et al., 2007), and parasitoids (Kindlmann & Ruzicka, 1992; Meyhöfer & Klug, 2002; Almohamad et al., 2008a). Apart from prey effects, intra-and interspecific competition may be an important factor regulating performance. Interactions between coexisting syrphid species that share the same aphid prey resource in a patchy habitat often result in intraguild predation, and larvae engage in conspecific and heterospecific predation of eggs and larvae (Benestad Hågvar, 1972; Branquart et al., 1997; Hindayana et al., 2001; Fréchette et al., 2007). The effects of such interactions in a guild may either lead to stabilizing of prey-predator populations (Godfray & Pacala, 1992) or adversely affect the foraging and oviposition performance of individual predators (Rosenheim et al., 1995; Agarwala et al., 2003).

Syrphid larvae are much less mobile than adults (Chandler, 1969). Additionally, several studies on intraguild predation among syrphid species and other predators have demonstrated that syrphid eggs and larvae are vulnerable to cannibalism (Branquart et al., 1997), and are highly susceptible to predation by other aphid predators such as the ladybird *Coccinella septempunctata* L., lacewing *Chrysoperla carnea* Stephens and gall midge *Aphidoletes aphidomyza* Rondani (Hindayana et al., 2001; Fréchette et al., 2007). Ovipositing hoverfly females would therefore benefit by developing an avoidance of intra- and interspecific individuals present in the same colonies in order to reduce the predation risk to their offspring. Recently it has been discovered that female aphidophages adapt their oviposition behavior in the presence of conspecific and heterospecific competitors. These studies have largely focused on chrysopids (Ruzicka, 1996), coccinellids (Doumbia et al., 1998; Agarwala et al., 2003) and the gall midge (Ruzicka & Havelka, 1998), but there are some on syrphids. A very good example is the study of Scholz & Poehling (2000) on *Episyrphus balteatus*, which demonstrated that ovipositing females avoid aphid colonies in which conspecific eggs are already present, and the oviposition-deterring stimuli were still active when the eggs were removed. Similar oviposition avoidance was shown by *Episyrphus balteatus* females to the presence of conspecific larvae (Völkl, 1990). Recent studies have demonstrated that the stimuli permitting this discrimination probably derive from syrphid eggs or larvae (Almohamad et al., unpublished data).

The presence of heterospecific competitors can influence foraging and oviposition. In the study of Almohamad et al. (2008a), foraging and oviposition behavior of *Episyrphus balteatus* females are affected by the presence of parasitoids: females laid significantly fewer eggs in colonies with mummified aphids than in unparasitized or parasitized colonies. They also showed oviposition avoidance response to the presence of *Harmonia axyridis* larvae (Almohamad et al., unpublished data). Thus the presence of intra-and interspecific individuals (i.e. intraguild predators) is likely to influence the choices made by ovipositing syrphids.

Effect of female syrphid age

Female age, through time limitation, may be an important factor determining a forager’s decision; when an organism is close to the end of its life it may be more advantageous for it to accept a poor quality oviposition site than it is for a young organism (Mangel, 1987). This decline in selectivity with age has much empirical support. For example, aphidophagous ladybirds *Adalia bipunctata* (L.) were less selective when older, or when they had previously experienced poor quality patches (Fréchette et al., 2004). Weisser (1994) demonstrated that the parasitoid *Lysiphlebus cardui* Marshall becomes less selective (for aphid age) as it ages. However, in the field, Heimpel et al. (1996) found no evidence that age affected the oviposition behavior of the parasitoid *Aphytis aonidiae* (Mercet).

The age effect is so general that it is incorporated into the hierarchy threshold model (Courtney et al., 1989; Sadeghi & Gilbert, 2000a,b), but the influence of age is not well-documented in aphidophagous hoverflies: so far we know of only three studies (Chandler, 1967; Guest, 1984; Sadeghi & Gilbert, 2000a). Young females of *Episyrphus balteatus* and *Syrphus ribesii* exhibit a marked hierarchical preference for particular species of aphids and do not oviposit on uninfested plants, but they lose discrimination as they get older (Sadeghi & Gilbert, 2000a); Guest (1984) showed that *Episyrphus balteatus* females increasingly lay eggs away from aphids as they age. In contrast, the distance between the nearest aphid and the egg decreased with female age in *Eupeodes luniger* (Chandler, 1967), and older female *Episyrphus balteatus* and *Syrphus ribesii* laid more eggs on uninfested plants than did young ones, indicating that ageing decreased responses to aphid-related stimuli more than to plant-related ones.

Effect of egg load and host deprivation

The hierarchy threshold model of host choice has two components: an inherent, fixed (in each individual) rank order of preference of hosts**,** and a variable threshold of acceptability that depends in part on internal factors such as egg load (i.e. the number of mature eggs in the ovaries) (Sadeghi & Gilbert, 2000c). This biological factor is found to be a source of variation in host choice by ovipositing females. Minkenberg et al. (1992) concluded that the role of egg-load, egg-load dynamics and the function of egg-load response will lead to a more complete understanding of variation in oviposition behavior. Host deprivation is also used to investigate the effect of the egg load on oviposition behavior (Fitt, 1986). Sadeghi & Gilbert (2000c) reported that *Episyrphus balteatus* and *Syrphus ribesii* (L.) females do not waste their mature eggs when facing a shortage of hosts or when there are no suitable aphids. Dixon (1959) also showed that female *Eupeodes corollae* could retain mature eggs in the absence of aphids, but eventually some eggs were laid. Females could retain mature eggs for several weeks in the absence of suitable oviposition site. Prolonged retention reduced fecundity but increased longevity (Lyon, 1965).

Conclusions

We conclude that several factors have been show to be involved in the selection of oviposition site by aphidophagous hoverflies. These factors include habitat, host-plant physical characteristics (i.e. floral characters), the aphid species, aphid colony size and density, semiochemicals emitted from aphids or their association with host plants, the presence of intra or interspecific competitors and female age. Females show evolved behavioral mechanisms in response to these factors that enable them to forage for an oviposition site that will support the development of their offspring. This review highlights much that has been learned, but also emphasizes that much remains to be learned about the mechanisms of decision-making by individual females to assess aphid patch quality during their egg-laying behavior. Detailed information about searching and oviposition behavior provides an essential foundation for designing effective biological control, and for better understanding when, where and how syrphids can suppress aphid populations.

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Chapter 3

Research Objectives

The ability of predatory hoverfly *E. balteatus* to detect aphids and oviposit close to aphid colonies is directly linked to its efficiency as biological control agent of aphids. Since syrphid larvae have limited dispersal abilities and only forage occasionally between areas of the plant with aphids, the female's oviposition decision is of crucial importance to the offspring. The selection of aphid patches by females should therefore reflect a preference for oviposition sites with high optimal conditions for offspring performance. Thereby it is important to understand the behavioural responses of decision-making by hoverfly females during egg-laying behaviour.

The main objective of the present thesis is therefore to identify the main factors which could be taken into account by hoverfly *E. balteatus* females in choice of their oviposition sites, as well as to better understand the cues and behavioural mechanisms of decision making by females during their eggs-laying behaviour, which enable them to locate and select a suitable oviposition site, taking into consideration that the 'quality' of aphid patch as an oviposition site may depends on several factors such as aphid species, aphid host plant, aphid numbers, semiochemicals and the presence of intra- or interspecific competitors.

The success of biological control efforts with aphidophagous predators was initially determined by two major factors, i.e. aphid prey suitability and ecological requirements of these antagonists. Aphidophagous hoverflies are likely to encounter different species of aphids or aphid associated with different host plants when foraging for oviposition site. However, aphid species or host plant-aphid combinations are not all equally suitable for larval growth or adult production (fitness). More detailed studies are therefore needed to evaluate the oviposition preference and larval performance of predatory hoverfly in response to different host plants or different aphid species. **<u>In the first part of present thesis</u>**, a series of experiments was conducted to determine the major factors (host plant and aphid species) that influence prey suitability for *E. balteatus* females in a tritrophic model: Solanaceae-aphids-predatory hoverfly. In the first experiment, the oviposition behaviour of *E. balteatus* was investigated in response to two host plants [*Solanum tuberosum* L. and *Solanum nigrum* L. (Solanaceae)] infested by one aphid species, *Myzus persicae* Sulzer (Homoptera: Aphididae). Indeed, hoverfly fitness calculations for different aphid host plants were based on larval and adult performance (development and reproduction parameters) and were related to oviposition behaviour. Secondly, we wanted to test the oviposition preference of *E. balteatus* females in response to aphid prey quality and its relation with syrphid fitness, by comparing different aphid species *Acyrthosiphon pisum* Harris, *Aphis fabae* Scopoli, and *Megoura viciae* Buckton (Homoptera: Aphididae), that infested the host plant *Vicia faba* L. (Leguminosae). In the third experiment, we wanted to assess the infochemical role of the aphid-infested plant volatiles on the aphidophagous predator *E. balteatus* foraging behaviour, in order to understand the mechanisms of indirect defense of aphid-infested host plants toward a predatory hoverfly when foraging for oviposition site.

Aphid colony size has an important influence on the selection of the oviposition site. Hoverfly females seem to be able to adjust their egg number to aphid density; a behaviour that may be considered as adaptive since it secures both larval survival and optimizes the female's searching effort. Generally, the number of eggs deposited increases with aphid colony size. However, the mechanisms as to how the predatory hoverfly females evaluate aphid colony with different sizes are uncertain. **In the second part of present thesis**, we wanted to understand the behavioural mechanisms of *E. balteatus* in response to different aphid colony sizes. Oviposition behaviour of hoverfly *E. balteatus* was first investigated in response to different aphid prey densities and also to different heights of aphid colony location using a leaf disc system. Secondly, we quantified the volatile organic compounds released in their headspaces from aphid *M. persicae* colony of different sizes. The behavioural impacts of these chemical cues released on decision–making processes that lead to oviposition were subsequently evaluated towards *E. balteatus* females.

Over the last two decades many experts assumed that intra-or interspecific interactions among aphid natural enemies can influence biological control efforts. Aphidophagous hoverflies can act as intraguild predators (IGP) against other aphid predators or parasitoids. Indeed, these intraguild interactions probably influence the choice of oviposition site made by predatory hoverfly female. More information are therefore needed to understand oviposition response of predatory hoverfly to these interactions. **In the third part of present thesis,** the behavioural reaction of hoverfly *E. balteatus* females was first investigated in response to the presence of conspesific larvae and their tracks in an aphid colony. Volatile chemical compounds of *E. balteatus* larval extracts were indentified and their role toward hoverfly females was also evaluated. Secondly, we tested the effects of tracks left by conspecific syrphid and heterospecific ladybird, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) larvae on the oviposition site discrimination by *E. balteatus* females. Oviposition response of *H. axyridis* females to tracks left by hoverfly larvae was also studied in one-choice experiments. We finally wanted to investigate the foraging and oviposition behaviour of *E. balteatus* in response to the presence of *Aphidius ervi* larvae parasitising the aphid colony. The effects of parasitised aphids as food on the fitness of *E. balteatus* larvae were also investigated.

In the fourth part of present thesis, we wanted to evaluate the influence of female's age on *E. balteatus* reproduction and to consider its use in biological control programs.

In the final part of present thesis, the results obtained were globally discussed and perspectives were proposed for additional experiments to complete our understanding of the behavioural mechanisms involved in oviposition decisions made by predatory hoverfly females.

Chapter 4

Tritrophic effects on oviposition preference and larval performance

In the previous chapter, the importance of predatory hoverfly E. balteatus in controlling the aphid populations was well documented. On the first hand, it has a significant impact on the suppression of aphid populations because of its high reproductive and voracity. On the other hand, E. balteatus females are known to exhibit high mobility, enabling them to distribute eggs over large areas, and to locate aphid colonies earlier in the season than other aphidophaga. The choice of an oviposition site by hoverfly females has therefore an important impact on offspring performance, because syrphid larvae have limited dispersal mobilities to forage for food and they do not perceive aphids before contact or only at short distance.

Before including E. balteatus in an integrated aphid management program, a good knowledge of hoverfly oviposition behaviour need to be acquired to understand the behavioural mechanism as to how females assess aphid patch quality. E. balteatus is a primary predator and belongs to the third trophic level in the food chain hierarchy. On this level aphid prey suitability as oviposition site can be affected not only by the aphid prey itself (direct effect), but also by the condition of the host plants (indirect effect). The present chapter aims to study the influence of major factors (host plant and aphid species) on the oviposition site selection by predatory hoverfly in a tritrophic model: Solanaceae-aphids-predatory hoverfly, and also to understand the infochemical role of semiochemicals which mediate these tritophic interactions. Two host plants [Solanum tuberosum L. and Solanum nigrum L. (Solanaceae)] and three aphid species [Acyrthosiphon pisum Harris, Aphis fabae Scopoli, and Megoura viciae Buckton (Homoptera: Aphididae)] were mainly used in the following experiments. Potato (S. tuberosum) is economically important crops. All aphids species studied are considered to be economical and important pests in many agricultural and horticultural ecosystems.

Article 2
Predatory hoverflies select their oviposition site according to aphid host plant and aphid species

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Abstract – The hoverfly *Episyrphus balteatus* De Geer (Diptera: Syrphidae) is an abundant and efficient aphidspecific predator. Several aphidophagous parasitoids and predators are known to respond positively to aphid-infested plants. Semiochemicals from the latter association usually mediate predator/parasitoid foraging behavior toward sites appropriate for offspring fitness. In this study, we investigated the effect of aphid host plant and aphid species on foraging and oviposition behavior of *E. balteatus*. Behavioral observations were conducted using the Noldus Observer v. 5.0, which allows observed insect behavior to be subdivided into different stages. Additionally, the influence of aphid species and aphid host plant on offspring fitness was tested in a second set of experiments. *Acyrthosiphon pisum* Harris and *Megoura viciae* Buckton were equally attractive for *E. balteatus* whereas *Aphis fabae* Scopoli (all Homoptera: Aphididae) were less attractive. These results were correlated with (i) the number of eggs laid, which was significantly higher for the two first aphid species, and (ii) the fitness of hoverfly larvae, pupae, and adults. Two solanaceous plant species, *Solanum nigrum* L. and *Solanum tuberosum* L. (Solanaceae), which were infested with *Myzus persicae* Sulzer (Homoptera: Aphididae), were also compared using the same approach. Discrimination between these two *M. persicae* host plants was observed, with *S. tuberosum* being preferred as an oviposition site by the predatory hoverfly. Larval and adult fitness was correlated with the behavioral observations. Our results demonstrated the importance of the prey–host plant association on the choice of the oviposition site by an aphid predator, which is here shown to be related to offspring fitness.

Key words: *Episyrphus balteatus*, oviposition behavior, Solanaceae, fitness, larval performance, Diptera, Syrphidae.

Reference – Almohamad R., Verheggen FJ., Francis F., Haubruge E. (2007). Predatory hoverflies select their oviposition site according to aphid host plant and aphid species. *Entomologia Experimentalis et Applicata.* **125** (3): 13–21.

Introduction

Natural enemies of herbivorous insects play an important role in the population dynamics of their prey (Price, 1987; Schoenly, 1990). In particular, the hoverfly, *Episyrphus balteatus* De Geer (Diptera: Syrphidae), is the most abundant in central Europe (Tenhumberg & Poehling, 1991; Colignon et al., 2001) and one of the most efficient aphid-specific predators in natural agroecosystems, particularly with respect to cereal aphids (Entwistle & Dixon, 1989; Tenhumberg & Poehling, 1995). Because syrphid larvae have limited dispersal abilities (Chandler, 1969), the choice of the oviposition site has an important impact on offspring performance.

Host-finding behavior of stenophagous aphid predators and parasitoids has been investigated intensively (Godfray 1994; van Alphen & Jervis, 1996). However, many of the recent studies were focused on coccinellids (Ferran & Dixon, 1993; Sengonça & Liu, 1994), while neglecting syrphids. Several factors were shown to impact the choice of the oviposition site for aphidophagous hoverflies: (i) the aphid species and their associated chemicals (Budenberg & Powell, 1992; Bargen et al., 1998; Sadeghi & Gilbert, 2000a,b); (ii) the host plant's physical and chemical characteristics associated with the aphid species (Dixon, 1958; Chandler, 1968a; Sanders, 1983; Vanhaelen et al., 2001); (iii) the aphid colony size and density (Kan, 1988; Scholz & Poehling, 2000; Sutherland et al., 2001); and (iv) the age of the female (Sadeghi & Gilbert, 2000c). Many semiochemicals, emitted either by prey or by their association with host plants, are presumed to play an important role in habitat selection by reducing the time needed for searching as well as increasing attack rates on prey (Dicke & Sabelis, 1988; Vet & Dicke, 1992).

Most insect species, including predators (Hodek, 1993), show specific food resource preferences (Schoonhoven et al., 1998). Therefore, the correlation between adult preference for particular oviposition sites and subsequent larval performance has been extensively studied in phytophagous species (Harris et al., 2001; Forister, 2004). However, only a small proportion of these studies established a link between oviposition preference and larval performance (Thompson, 1988; Mayhew, 2001). Takeuchi et al. (2005) found that the phytophagous ladybird *Epilachna admirabilis* Crotch showed no preference between *Trichosanthes cucumeroides* Maxim and *Gynostemma pentaphyllum* Makino, even though the larvae performed better on the first plant species.

According to Gilbert (2005), there are few studies concerning the oviposition preference of female aphidophagous syrphids and larval performance towards different host plants or different aphid species. In this study, the oviposition behavior of *E. balteatus* was investigated for two host plants [*Solanum tuberosum* L. and *Solanum nigrum* L. (Solanaceae)] infested by one aphid species, *Myzus persicae* Sulzer (Homoptera: Aphididae), in a dual-choice experiment. Hoverfly fitness calculations for different aphid host plants were based on larval and adult performance (development and reproduction parameters) and were related to oviposition behavior. Additionally, similar experiments were conducted with three aphid species, *Acyrthosiphon pisum* Harris, *Aphis fabae* Scopoli, and *Megoura viciae* Buckton (all Homoptera: Aphididae), that infested *Vicia faba* L. (Leguminosae). Lastly, we investigated oviposition behavior and larval and adult performances.

Materials and methods

Plant and insect rearing – Broad beans (*V. faba* L.) and the two Solanaceae plants (*S. tuberosum* L. and *S. nigrum* L.) were grown in 30x20x5 cm plastic trays filled with a mix of compost, perlite, and vermiculite (1:1:1) and maintained in controlled environment growth rooms (L16:D8 and 20 ± 1°C). Six-leaf solanaceous plants were used in the following experiments. *M. persicae* was reared on *V. faba*, *S. tuberosum*, and *S. nigrum* in separate controlled temperature rooms set at the same conditions as described above. The other aphid species, *M. viciae*, *A. pisum*, and *A. fabae*, were taken from stock cultures grown on *V. faba*. Adult *E. balteatus* were reared in 75x60x90 cm cages and were fed with bee-collected pollen, sugar, and water. Broad beans infested with *M. viciae* were introduced into the cages for 3 h every 2 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 a standard diet.

Oviposition preference

***Aphid host plant preference* –** In two-choice experiments, females were placed individually in net cages (30 × 30 × 60 cm) with two host plants infested with 400 *M. persicae* (*M. persicae*/*S. tuberosum* vs. *M. persicae*/*S. nigrum*). Their foraging behavior was then recorded for 10 min using the Observer® software (Noldus information Technology, version 5.0, Wageningen, The Netherlands). Descriptions of the four observed behavioral subdivisions are presented in Table 3. In similar two-choice experiments (*M. persicae*/*S. tuberosum* vs.*M. persicae*/*S. nigrum*), a single *E. balteatus* female was allowed to lay eggs for 3 h and the number of eggs laid on each aphid host plant was counted. The experiments were conducted in a controlled temperature room at 20 ± 1°C. *E. balteatus* females were approximately 20–30 days old and no induction of oviposition had been realized for 24 h prior to the experimentation. There were 10 replicates for each of the aforementioned experiments.

Aphid species preference – In similar two-choice experiments, a single *E. balteatus* female was placed in a cage with two *V. faba* plants (with six leaves and 20 cm high), which were infested with different aphid species. Three combinations of aphids species were used (*A. pisum* vs. *M. viciae*, *A. pisum* vs. *A. fabae*, and *M. viciae* vs. *A. fabae*). By using the Observer® recorder, the behavioral subdivisions of the female hoverfly were recorded for 10 min and were observed to be identical to earlier tests (Table 3). In similar two-choice experiments (*A. pisum* vs. *M. viciae*, *A. pisum* vs. *A. fabae*, and *M. viciae* vs. *A. fabae*), a single *E. balteatus* female was allowed to lay eggs for 3 h and the number of eggs laid (oviposition rate) on each infested plant was counted. Experiments were conducted in a controlled temperature room at 20 ± 1°C. *E. balteatus* females were approximately 20–30 days old and no induction of oviposition had been realized for 24 h prior to the experiment. Eight replicates for each pair of aphid species were performed.

Table 3. Description of the behavioral events recorded for aphidophagous hoverfly *E. balteatus* exposed to different host plants of prey aphid.

Observed behavior		**Description of behavior**
Immobility/ cage		Predator immobilizes on the cage
Searching	Fly/cage	Predator flies in the cage
	Fly/plant	Predator flies near the plant
Acceptance of host plant	Immobile/plant	Predator landing on the plant
	Walking/plant	Predator moving on the plant
	Immobile proboscis/plant	Predator extends its proboscis and identifies the stimulatory substrate to accept the host.
	Walking proboscis/plant	
Oviposition behavior	Immobile abdomen/plant	Predator exhibits an abdominal protraction or oviposition**.**
	Walking abdomen/plant	
	Egg laying	Oviposition

Larval performance

Effect of aphid host plant –To assess the effect of aphid host plants on the fitness of *E. balteatus*, 30 newly emerged first instars were weighed and individually placed in plastic Petri dishes (9 cm in diameter). Each day, the larvae were fed an excess of *M. persicae*, which was taken from either of the host plants (*S. tuberosum* or *S. nigrum*). Hoverfly larvae were kept in an incubator at 20 ± 1°C and L16:D8, and the developmental time and survival rates were determined. The pupae and the adults were also weighed (using a Sartorius microbalance scale model Mc5) and placed, in male/female pairs, in 60×30×30 cm net cages. Fecundity and egg viability of female hoverflies were recorded daily during 3 weeks. Individual fitness (r) was calculated as a performance measure (McGraw & Caswell, 1996) by integrating developmental time (D), survival (m = 1 or 0), and potential fecundity (*V*) using the equation: $r = [\mathrm{Ln}\,(m\cdot V)]/D$, where Ln is the natural logarithm.

Effect of aphid species –To assess the effect of the consumed aphid species on *E. balteatus* fitness, 30 newly emerged first instars were weighed and individually placed in plastic Petri dishes (9 cm in diameter). Each larva was fed an excess of each aphid species daily. This experiment was conducted with the three following aphid species: *A. pisum*, *M. viciae*, or *A. fabae*. The Petri dishes were kept in a controlled temperature room at 20 ± 1°C and L16:D8, and the developmental time and survival rates were determined. The pupae male/female the adults were also weighed and placed in male and female pairs in 60 × 30 × 30 cm net cages. Fecundity and viability of eggs were recorded daily during 3 weeks. Individual fitness (r) was calculated as presented above (McGraw & Caswell, 1996).

Statistical analysis

Means were compared using one-way analysis of variance (ANOVA) and Student's t-test or Tukey's test, conducted with Minitab® software (version 12.2, Minitab Inc, State College, PA, USA). Observed frequencies related to the adult emergence rates were compared to the corresponding frequencies from the control using $\chi 2$ tests. Percentage of mortality and egg viability were transformed using the angular transformation before ANOVA (arcsine $\sqrt{x}$; Dagnelie, 1973).

Results

Oviposition preference

Aphid host plant preference – In the dual-choice experiment, a significant preference of female hoverflies for the *M. persicae*-infested *S. tuberosum* was observed (Figure 2).

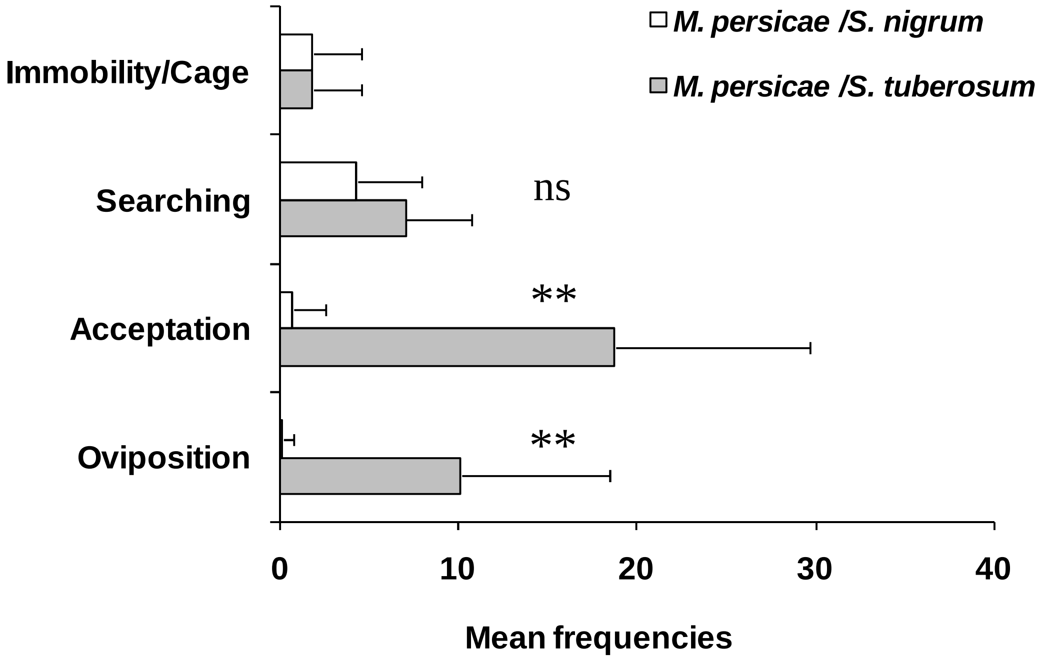

Figure 2. Behavioral observations (mean frequencies ± SD) on the oviposition pattern of *E. balteatus* females in relation to aphid host plants in two-choice experiment; ns and ** indicate no significant and significant differences at P < 0.01 (n = 10).

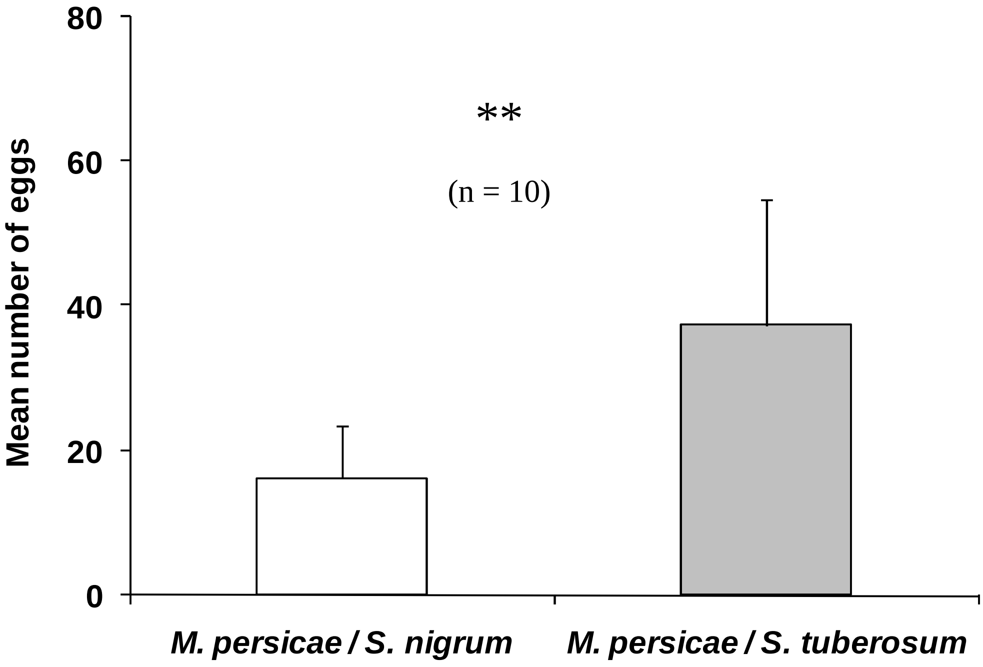

Figure 3. Effects of aphid host plants on oviposition rates (mean number of eggs ± SD) of *E. balteatus* in two-choice experiments after 3-h exposure with *Myzus persicae* as prey and *Solanum nigrum* and *Solanum tuberosum* as host plants. ** indicates significant diffrences at P < 0.01.

S. tuberosum induced higher frequencies of acceptance (landing, walking, and proboscis extension) (Student's t-test: t = 5.17, P = 0.001) and oviposition (Student's t-test: t = −3.71, P = 0.005) by the hoverfly**.** In addition, the number of eggs laid by *E. balteatus* females was significantly affected by the aphid host plant species with *S. tuberosum* being significantly preferred as an oviposition site (Student's t-test: t = −3.54, P = 0.004) (Figure 3).

Aphid species preference – Whereas no significant difference in terms of hoverfly acceptance behavior was observed between *A. pisum*- and *M. viciae*-infested broad beans plants (Student's t-test: t = 1.33, P = 0.226), female hoverflies prefer, and lay eggs on, one of the two aforementioned aphid species rather than on *A. fabae*-infested plants (Figure 4).

Female *E. balteatus* significantly preferred landing and laying eggs on *A. pisum*-infested broad beans rather than on the same host plant infested with *A. fabae* (Student's t-test: t = 2.64, P = 0.033). *M. viciae*-infested broad beans were also significantly preferred to *A. fabae*-infested broad beans in terms of host plant acceptance and oviposition site (Student's t-test: t = 3.62, P = 0.014).

These behavioral preferences were correlated with the number of observed eggs on the host plant of the aphid species (Figure 5). Similarly to the previously presented results, no significant preference was observed between *M. viciae* and *A. pisum* (Student's t-test: t = −0.47, P = 0.648). These two aphid species corresponded with the more suitable species to induce egg oviposition by female hoverflies. Indeed, these females laid fewer eggs on *A. fabae*-infested broad beans than on *M. viciae* (Student's t-test: t = 3.90, P = 0.001) or *A. pisum*-infested plants (Student's t-test: t = 6.22, P < 0.001).

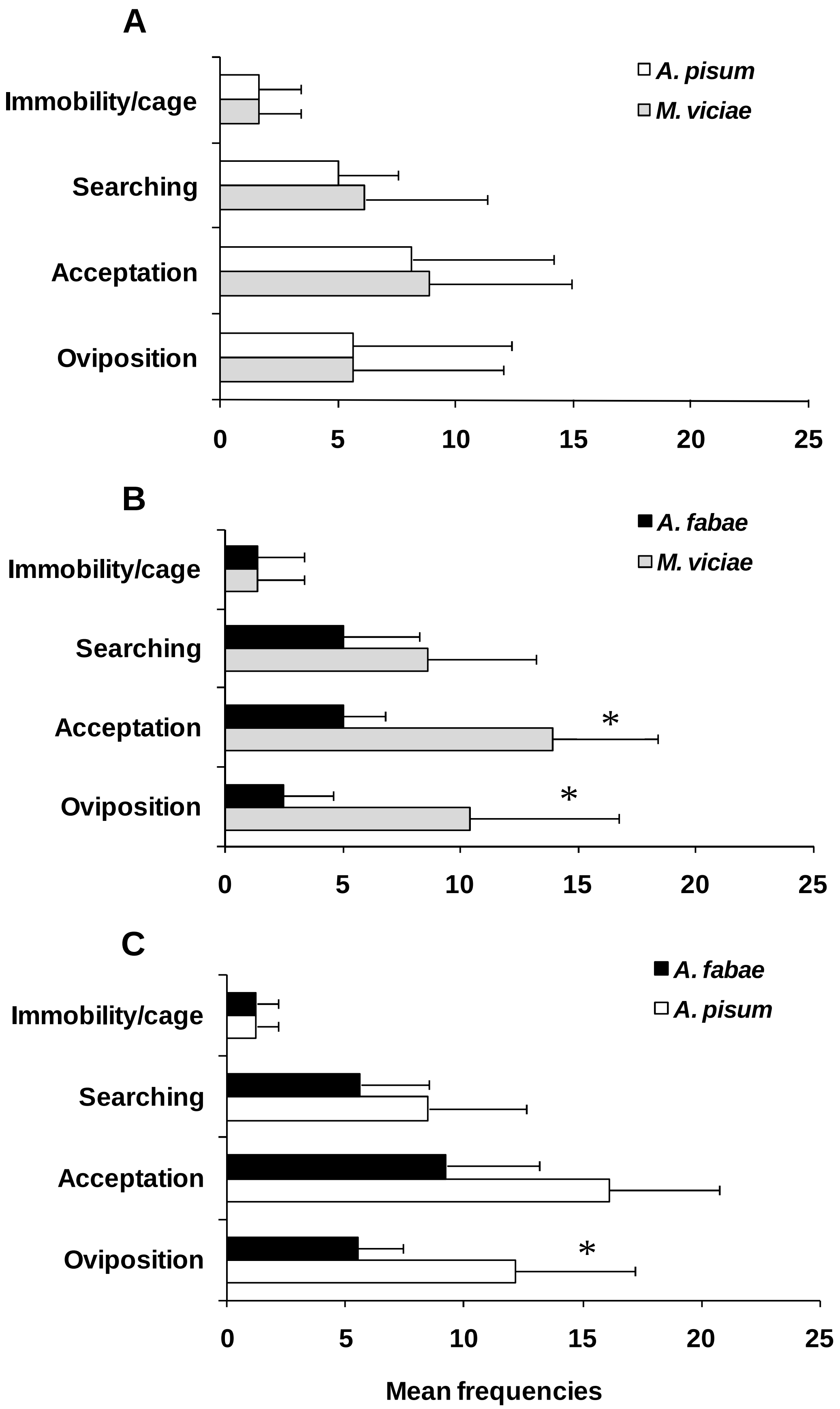

Figure 4. Behavioral observations (mean frequencies ± SD) on the oviposition pattern of *E. balteatus* adults in relation with aphid species in two-choice experiment; an * indicates significant differences at $P < 0.05$ ($n = 10$).

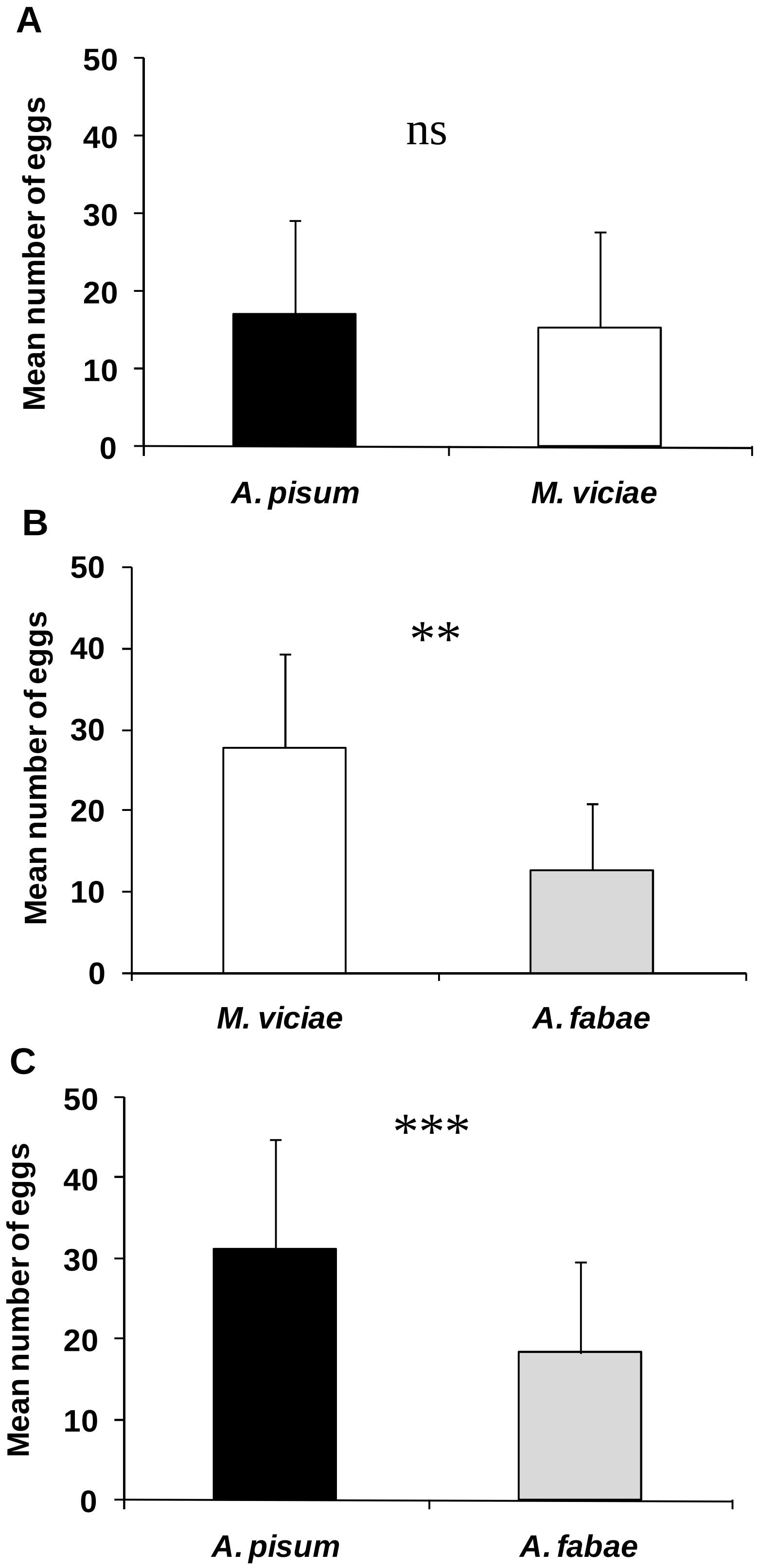

Figure 5. Effects of aphid species on oviposition rates (mean number of eggs ± SD) of *E. balteatus* in two-choice experiment after 3h exposure; ns, **, and * indicate no significant and significant differences at $P < 0.01$ and $P < 0.001$, respectively, (n = 8).**

Larval performance

Effect of aphid host plant – Several parameters concerning the larval, pupal and adult development of *E. balteatus* have been compared for hoverflies fed with the same aphid species (*M. persicae*) but reared on two different host plants (*S. tuberosum* and *S. nigrum*). (Table 4). No difference in survival of larvae and adults was observed ($\chi^2 = 0.00$, d.f. = 2, P = 1.00 and $\chi^2 = 0.073$, d.f. = 2, P = 0.964, respectively). However, larvae fed on *M. persicae* infesting *S. tuberosum* needed less time to reach the pupal stage (t = - 3.95, P<0.001). The resulting pupae were significantly heavier (t = 2.66, P = 0.012), the time required to reach the adult stage was significantly shorter (t = - 4.32, P < 0.001) and no difference in adult weight was observed (t = 1.82, P = 0.077). Although hoverfly fecundity (eggs/female/day) and egg viability did not differ significantly according to solanaceous host plant (t = 1.22, P = 0.223 and t = 0.29, P = 0.775, respectively), hoverfly fitness (r) was significantly higher on *M. persicae/S. tuberosum* rather than on *M. persicae*/*S. nigrum* (t = 2.45, P = 0.040).

Table 4. Effect of aphid host plant on the developmental and the reproductive performance of predatory hoverfly *E. balteatus*. (Mean ± SD). Significant, grand significant differences and high significant differences at P < 0.05, P < 0.01 and P < 0.001 respectively.

Biological parameters	***Myzus persicae*/ host plants**		**Test statistic**	
	S .tuberosum	***S. nigrum***		
Larval development (days)	7.75 ± 0.79	8.81 ± 0.93	t = - 3.95	P < 0.001
Survival of larvae (%)	66.66 ± 9.19	66.66 ± 9.19	$X^2_2 = 0.00$	P = 1.000
Survival (%) (to adult emergence)	63.33 ± 4.67	66.66 ± 9.19	$X^2_2 = 0.07$	P = 0.964
Pupal weight (mg)	35.16 ± 4.41	31.06 ± 0.93	t = 2.66	P = 0.012
Pupal development (days)	7.53 ± 0.51	8.35 ± 0.67	t = -4.32	P < 0.001
Adult weight (mg)	22.01 ± 2.78	20.46 ± 2.53	t = 1.82	P = 0.077
Egg to adult develoment (days)	18.74 ± 1.66	20.20 ± 0.77	t = -3.50	P = 0.002
Pre-oviposition duration	9.66 ± 2.81	9.20 ± 1.79	t = 0.33	P = 0.747
Fecundity, egg/♀/day	30.83 ± 31.25	25.25 ± 27.35	t = 1.22	P = 0.223
Total egg viability,%	81.29 ± 7.91	80.93 ± 5.24	t = 0.29	P = 0.775
Fitness (r)	0.78 ± 0.08	0.65 ± 0.09	t = 2.45	P = 0.040

Effect of aphid species – Differences in *E. balteatus* developmental parameters by aphid species (*M. viciae*, *A. pisum*, and *A. fabae*) were observed (Table 5) and *E. balteatus* larvae developed to maturity with each of the aphid species tested. Additionally, there was no significant difference in larval survival rates, which ranged from 73.33% for *A. fabae* to 80.00% for *A. pisum*. However, the aphid species significantly influenced the time needed for the larvae to reach pupal stage, where days required ranged from 8.85 (*A. pisum*) to 9.86 (*A. fabae*) ($F_{2,65} = 19.88$, $P < 0.001$). The pupae on a diet of *A. fabae* were significantly lighter ($F_{2,65} = 8.55$; $P \leq 0.001$) and needed more time to reach the adult stage ($F_{2,65} = 4.84$; $P \leq 0.011$). In terms of the egg to adult development time, the time required on *A. fabae* was significantly greater, reaching 17.81 days whereas only 15.50 days were needed for the individuals reared on *A. pisum*.

Table 5. Influence of the aphid species on various performance parameters of development of *E. balteatus*. (Mean ± SD). Significant, grand significant differences and high significant differences at P < 0.05, P < 0.01 and P < 0.001 respectively. Different letters indicate significant diffrences between treatments.

	Aphid prey species				
Biological parameters	***M.viciae***	***A. pisum***	***A. fabae***	**Test statistic**	
Larval development (days)	9.17 ± 0.39**a**	8.58 ± 0.65**a**	9.86 ± 0.91**b**	$F_{2, 65} = 19.88$	$P < 0.001$
Survival of larvae (%)	76.66 ± 14.14a	80.00 ± 9.62a	73.33 ± 9.48a	$\chi^2_2 = 0.37$	$P = 0.830$
Survival (%) (to adult emergence)	73.33 ± 18.81a	73.33 ± 18.81a	66.67 ± 9.40a	$\chi^2_2 = 0.43$	$P = 0.805$
Pupal weight (mg)	31.82 ± 3.55ab	34.49 ± 4.10a	29.69 ± 4.03b	$F_{2,65} = 8.55$	$P \leq 0.001$
Pupal development (days)	7.09 ± 0.41ab	6. 88 ± 0.45a	7.29 ± 0.46b	$F_{2,65} = 4.84$	$P \leq 0.011$
Adult weight (mg)	19.85 ± 2.51a	22.45 ± 3.55b	18.66 ± 3.67a	$F_{2,61} = 7.45$	$P \leq 0.001$
Egg to adult development (days)	16.35 ± 1.81a	15.50 ± 0.72a	17.81 ± 1.81b	$F_{2,65} = 18.14$	$P < 0.001$
Pre-oviposition duration	10.33 ± 0.52 a	9.20 ± 1.30a	10.40 ± 0.55a	$F_{2,13} = 3,25$	$P = 0.072$
Fecundity, egg/♀/day	32.11±32.75a	34.43 ± 32.77a	27.06 ± 36.02a	$F_{2, 221} = 0.87$	$P = 0.419$
Total egg viability,%	77.46 ± 11.54a	72.79 ± 10.60a	74.53 ± 16.16a	$F_{2, 157} = 2.03$	$P = 0.135$
Fitness (r)	0.66 ± 0.06ab	0.70 ± 0.06a	0.58 ± 0.08b	$F_{2,13} = 4.69$	$P = 0.029$

The fecundity (eggs/female/day) and egg viability were not significantly influenced by the aphid species *M. viciae, A. pisum,* and *A. fabae* ($F_{2,221}$ = 0.87, P = 0.419; $F_{2,157}$ = 2.03, P = 0.135, respectively). However, *E. balteatus* female fitness (r*)* was significantly higher on broad beans infested with *A. pisum* or *M. viciae* than on *V. faba* infested with *A. fabae* ($F_{2,13}$ = 4.69, P = 0.030).

Discussion

In this study, the effect of the aphid host plant variety on the choice of oviposition site by *E. balteatus* was clearly demonstrated. Sadeghi & Gilbert (2000 a,b) highlighted the ability of hoverflies to discriminate their potential oviposition sites, which consisted of aphid species and their associated host plant. However, these authors could not conclude whether the attraction and oviposition induction was due to the aphid species, the host plant, or the interaction of host plant and prey. Using the same aphid species (*M. persicae*), reared on both *S. tuberosum* and *S. nigrum*, we were able to compare the effect of the host plant on the oviposition site preference and hoverfly fitness. Indeed, *S. tuberosum* induced higher frequencies of acceptance and received more eggs from hoverfly females than *S. nigrum*. In addition, the global hoverfly fitness was higher with *M. persicae* fed on *S. tuberosum,* confirming the hypothesis that ovipositing insects can select sites that improve the growth and survival of their offspring (Peckarsky et al., 2000). This should be even more true for insects that are unable to migrate easily from habitats poor in food, such as syrphid larvae. The reason behind the preference of *E. balteatus* for one plant rather than the other remains uncertain. When predators attempt to locate the prey habitat, they often use odors associated with prey presence, such as those from the herbivorous prey itself (Whitman, 1988), or from prey by-products such as feces or honeydew (Budengerge & Powell, 1992; Scholz & Poehling, 2000; Francis et al., 2004). Moreover, predators can use volatiles that are produced by plants in response to herbivore damage, such as “green” alcohols and aldehydes (Al Abassi et al., 2000; Francis et al., 2001). For example, Obata (1986, 1997) suggested that the Asian ladybird *Harmonia axyridis* Pallas was more strongly attracted to the odor of aphid-infested plants than to those of uninfested plants, and the volatile profiles of the two host plants are indeed different. Apart from the aphid-released (*E*)-β-farnesene, *S. tuberosum* release important amounts of the aphid alarm pheromone (Agelopoulos et al., 2000) whereas *S. nigrum* does not release this sesquiterpene (Schmidt et al., 2004). The (*E*)-β-farnesene was shown to attract predators such as *E. balteatus* (Francis et al., 2005b) which may explain the preference of female hoverflies for *S. tuberosum*. Plant color is one of the many stimuli used by phytophagous insects to recognize their host plant (Kelber, 2001). Sutherland et al. (1999) also demonstrated that aphid host plant color influenced the foraging behavior of the predatory hoverfly *E. balteatus*. However, this parameter could not explain the differences we obtained, as both plants (*S. tuberosum* and *S. nigrum*) were of similar color.

Our results also confirm the statement that hoverflies choose their oviposition site according to the infesting aphid species. Indeed, we demonstrated that *A. fabae* was not as attractive for *E. balteatus* as *A. pisum* and *M. viciae.* Our data are also in accordance with those of Sadeghi & Gilbert (2000a), who showed the pea aphid to be preferred among eight aphid species. However, *M. viciae* and *A. fabae* were not tested. The size of the aphid species tested might be a factor of importance in host selection. Indeed, similar numbers of aphids were tested, but whereas *A. pisum* and *M. viciae* are large aphids, *A. fabae* is slightly smaller and therefore represented less food for hoverfly offspring. The three tested aphid species release (*E*)-β-farnesene (Francis et al., 2005a) but might not release similar quantities, which could be specific or size dependent. The oviposition stimulus can also come from the aphid honeydew (Bargen et al., 1998; Scholz & Poehling, 2000), which varies qualitatively and quantitatively from one species to another and during the season (Fischer & Shingleton, 2001; Wool et al., 2006). Data vary from one predator to another. For example, even when reared on the same host plant (*V. faba*), the pea aphid (*A. pisum*) is considered suitable and the black bean aphid (*A. fabae*) is moderately suitable for larval development of the two-spotted ladybird *Adalia bipunctata* L. (Rana et al., 2002; Fréchett et al., 2006). However, the vetch aphid was found to be highly toxic for the same species (Fréchett et al., 2006).

The concordance between oviposition site selection and offspring performance is complex (Janz et al., 1994). Observed relationships between adult preference and some components of larval performance range from good concordance (Singer, 1983; Rausher, 1982) to poor concordance (Courtney, 1981). In some cases, poor concordance between preference and performance may result from oviposition onto introduced host plants (Chew, 1977; Legg et al., 1986) or relative rarity of the preferred host (Williams, 1983). Chandler (1968b) showed that the selection of an adequate oviposition site by syrphid females that lay eggs close to aphid colonies is essential to ensure the survival and fast development of their offspring. In our work, *E. balteatus* females demonstrated variations in their oviposition preference among the three tested aphids or among the two host plants and these differences had important consequences for the performance of their offspring.

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Article 3

Role of terpenes from aphid-infested potato on searching and oviposition behaviour of *Episyrphus balteatus*

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Abstract – To cope with pathogen and insect attacks, plants develop different mechanisms of defence, in both direct (physical and chemical) and indirect ways (attractive volatiles to entomophagous beneficials). Plants are then able to express traits that facilitate “top-down” control of pests by attracting herbivore predators. Here we investigate the indirect defence mechanism of potato plants by analyzing the volatile patterns of both healthy and aphid infested plants. Important changes in the emitted terpene pattern by the *Myzus persicae* infested host plant were observed. Using Solid Phase Micro Extraction (SPME) and GC-MS, the (*E*)-β-farnesene (EβF) appeared to be emitted by aphid-infested potato and not by healthy plants. To assess the infochemical role of these volatile releases after aphid damage on the aphidophagous predators *Episyrphus balteatus*, the hoverfly foraging behavior was assessed using the Observer 5.0 software (Noldus, Wageningen, The Netherlands). Aphid free potato plants were also used as a control volatile source in the predator behavioral study. While aphid-infested plants induced efficient searching and acceptation behaviors leading to egg-laying, no kairomonal effect of healthy potato plants was observed, leading to longer immobility durations and shorter searching periods in the net cage. High oviposition rate of *E. balteatus* was observed when aphid-infested potato was used (mean of 48.9 eggs per laying and per female). On the other hand, no egg was produced by the hoverfly on healthy aphid-free plants. The *E. balteatus* foraging and reproductive behaviors according to the volatile emission from aphid-infested plants are discussed in relation to the potential use of active infochemical molecules in integrated aphid pest management.

Key words: Aphid infested, behavior, *Episyrphus balteatus*, potato, terpenes

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Introduction

Plant-insect relations are mainly regulated by the evolution of the plant defence mechanisms and the ways herbivorous insects adapt themselves to these defensive systems (Berenbaum, 1995). A broad range of insect pests is efficiently controlled by the production of defensive molecules. Beside the induction of several direct defence molecule productions, such as secondary compounds and pathogenesis related proteins (PRP), the emission of particular volatile organic compounds (VOCs) constitutes an efficient indirect system of plant defence by influencing the third trophic level, namely the entomophagous beneficials. In tritrophic interactions studies investigating plant-hervivore-entomophagous insect relations, the plant response to herbivore damage has already been shown to affect the biological parameters of beneficial species (Kessler & Baldwin, 2001). In particular, the aphid-host plant species was shown to significantly affect ladybird and hoverfly development and reproduction (Francis *et al.*, 2001; Vanhaelen *et al.*, 2002). Not only the biological parameters were affected by aphid and host plant associations but the behaviour of beneficial also. Recently, reports of electroantennogram (EAG) recordings from three predatory insect species, namely *Coleomegilla maculate* DeGeer (Coleoptera, Coccinellidae), *Chrysoperla carnea* Stephens (Neuroptera, Chrysopidae) (Zhu *et al.*, 1999), and *Coccinella septempunctata* L. (Coleoptera, Coccinellidae; Al Abassi *et al.*, 2000) showed significant EAG responses to semiochemicals released from potential preys and host plants (Zhu & Park, 2005). These predators possibly use such chemicals to locate their prey. Ninkovic *et al.* (2001) also demonstrated that the seven-spot ladybird, *C. septempunctata*, responded positively to volatiles from the aphid, *Rhopalosiphum padi* L., and *Hordeum vulgare* L. infested plants. Two molecules, namely (*E*)-*β*-farnesene (EβF) and *β* -caryophyllene, were found to be a kairomone and an informative inhibitor respectively, for the seven-spot ladybird by electroantennography and olfactometry methods (Al Abassi *et al.*, 2000). The release of plant VOCs, notably the terpenoids, specifically after herbivory, are known to attract parasitoids and predators. These herbivore-induced VOCs actively increased the feeding activity of entomophagous larvae and global predation pressure on the herbivores (De Moraes *et al.*, 1998). Particularly, (*Z*)-3-hexen-1-ol, linalool and (*Z*)-*α*-bergamotene from herbivore-damaged plants were found to attract predators and to increase the predation rate by generalist predators (Kessler & Baldwin, 2001).

Particularly, the *Episyrphus balteatus* hoverfly positively responded to semiochemical cues from preys, from host plants and aphid-host-plant associations: *E. balteatus* was attracted by EβF, the well known aphid alarm pheromone (Francis *et al.*, 2005b). The latter molecule was identified as the main volatile substance in 16 species, alone or associated with other molecules (Francis *et al.*, 2005a). Also, the alarm pheromone was only a minor component of the volatile molecule patterns from five other aphid species. Only two of the 23 tested species, *Euceraphis punctipennis* (Zetterstedt) and *Drepanosiphum platanoides* (Shrank), did not release EβF at all but other terpenes were identified (Francis *et al.*, 2005a). Terpene molecules were found to be released by aphids but also by plants, mainly under different stress situations such as after pest damage (Francis *et al.*, 2001). Particular volatile emissions from aphid-plant associations could be then used as reliable cues by aphidophagous beneficials to locate potential preys. Investigation of the volatile pattern variations of aphid-infested plants and the assessment of their infochemical role is necessary to better understand the relations between plants, aphids and the aphidophagous beneficials. The objective of this work was to understand mechanisms of indirect defence of aphid-infested potato plants toward a predatory beneficial, namely the *E. balteatus* hoverfly, by: (i) collecting the volatile pattern of healthy and *Myzus persicae* infested plants by SPME and analyzing them by GC-MS; and (ii) by assessing the infochemical role of the aphid-infested plant volatiles on the aphidophagous predator *E. balteatus* foraging behavior by using the Observer 5.0 software (Noldus).

Materials and methods

***Insect and plant rearing* –** Potatoes (*Solanum tuberosum* L.) were grown in 30 cm ×20 cm ×5 cm plastic trays including a mixture of vermiculite and perlite (1/1) and were used as host plants for *Myzus persicae* Sulzer. Aphids were reared in a condition- controlled room (16: 8 L: D and 20 ± 2°C).

Adults of *Episyrphus balteatus* were reared in net cages (75 cm × 60 cm × 90 cm). The latter were fed with pollen and sugar in separate Petri dishes. Water was also supplied. The rearing was realised at 20 ± 2oC and 16: 8 L: D. Two-to- three-week-old hoverflies were used in the following experiments. At hatching, *E. balteatus* larvae were placed in aerated plastic boxes (11 cm ×10 cm ×4 cm) to be fed with *M. persicae ad libidum*. Hoverflies were mass-reared in a condition-controlled room under identical environmental conditions as previously described.

***Volatile collection* –** The last leaf (composed of 5 leaflets) was picked from 36-day-old potato plants *S. tuberosum* c.v. Bintjes and immediately used for volatile emission analysis. Three different samples were prepared:

Sample 1. Intact leaf was cut and introduced in a 10 ml septum-cap vial and allowed to equilibrate for 10 min at 40°C.

Sample 2. Leaves were individually pricked with 9 entomological pins and were then placed in a vial and allowed to equilibrate as above.

Sample 3. Leaf was infested for 7 days with 50 *M. persicae* aphids before cutting and preparing as above. Each condition was analyzed in triplicate. Volatiles from each sample were collected by SPME technique. Supelco SPME devices coated with divinylbenzene/ carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/ 30 µm) were used to sample the potato leaf headspace. After the equilibration time, the fiber was exposed to the headspace for 50 min at 40°C.

***Volatile analysis by GC-MS* –** The volatiles sampled by SPME were analyzed after equilibration time by GC-MS on an Agilent 5973 mass selective detector (MS), scanning from m/z 35-350, coupled with an Agilent 6890N gas chromatograph. The following analytical conditions were used: splitless injection at 250°C, HP5-MS (5% phenyl-dimethylpolysiloxane) column (30 m x 0.25 mm, df = 1 µm). The temperature program was from 35°C (2 min hold) to 150°C at 5°C/min then to 260°C at 20°C/min. Injector temperature was 270°C. The MS spectra were obtained in the EI mode at 70eV. The analytes were identified on the basis of their retention times and by interpretation of MS fragmentation patterns. The recorded spectra were finally compared to: (i) those of the Wiley238.L spectral library; and (ii) those related to previous analysis of our pure terpene references.

***Behavioral assays* –** Free or aphid-infested *S. tuberosum* plants (6 true leaves, 20 cm high, 36-day-old plants) were presented to *E. balteatus* gravid female (10 replicates per assay) in a no-choice presence of either healthy or aphid-infested plants and was observed for 10 min. The behaviors that were visually observed and simultaneously encoded using the Observer 5.0 software (Noldus) were grouped as described below:

1. Immobility: when the hoverfly stayed on the net cage without moving;
2. Searching: when the syrphid flew in the cage around the infested plant, either in an extensive or intensive way;
3. Acceptation: when the hoverfly landed on the plant, stayed immobile or walked on it, made proboscis extension on the plant surface;

4. Oviposition: when the syrphid had abdomen-bending and laid eggs.

Reproduction efficacy study – Hoverfly oviposition efficacy was observed by providing either aphid-infested or healthy plants in the net cage (similar to the ones previously described). A single *E. balteatus* female was introduced in the cage including one (aphid infested or healthy) plant for 3 hours. The eggs on plants were counted after this period.

Statistical analysis

The hoverfly behavior in the net cage was analyzed by pairwise mean comparison tests using Minitab software (12.2 version). Predator reproductive efficacies were analyzed using mean comparison tests according to the Tukey method.

Results

Even if *β*-caryophyllene was the main volatile compound emitted by both healthy and mechanically damaged potato plants (71.6% ± 6.7% and 68.8% ± 4.9% of the total volatile emission respectively), this relative abundance decreased to 48.2% ± 5.3% when the potato plants were infested with *M. persicae* aphids. Proportions of other volatile compounds according to the different plant states are presented in Figure 6. Healthy plants emitted nine volatiles as did mechanically damaged ones. The volatile pattern from aphid-infested plants was more diversified, including five supplementary volatiles, namely the *β*-sesquiphellandrene, the EβF, the *α*-zingiberene, the *β*-bisabolene and the germacrene D-4-ol. Due to the similar volatile patterns emitting from both healthy and mechanically damaged plants, only healthy plants were compared to aphid-infested ones. The infochemical role of aphid-infested plants was observed on the different behavioral groups (Figure 7). First, *E. balteatus* was significantly less immobile in the net cage in the presence of aphid-infested plants (t = 2.93, P = 0.011). Second, the searching frequencies related to the *M. persicae* infested potato was twice as high as the ones observed with healthy plants (t = 2.00, P = 0.05). The following step in the host-plant-prey selection, namely the acceptation, was significantly higher for the syrphid female in contact with aphid-infested than for healthy plants (t = 4.05, P = 0.002). Finally, the predator oviposition was significantly higher when *M. persicae* infested rather than healthy plants were used in the net cage (t = 3.23, P = 0.010). Hoverfly was shown to be receptive to the indirect defence cues from aphid-infested potato plants. While the female hoverfly mobility already increased in the first few minutes in the net cage, oviposition, acceptation and research frequencies were obviously induced in the presence of aphid-infested potatoes. To quantify the *E. balteatus* reproductive efficiency, a last parameter was observed to complete the predatory hoverfly foraging assessment; reproductive behavior was investigated, that is, the predator egg number laid on the plants. As a mean of 48.9 ± 21.2 eggs per laying and per female was observed on aphid-infested plants, and no egg was produced by the hoverfly on healthy aphid-free plants (Figure 8), a highly significant difference was observed according to the presence of *M. persicae* on plants ($t = 6.87$, $P < 0.001$).

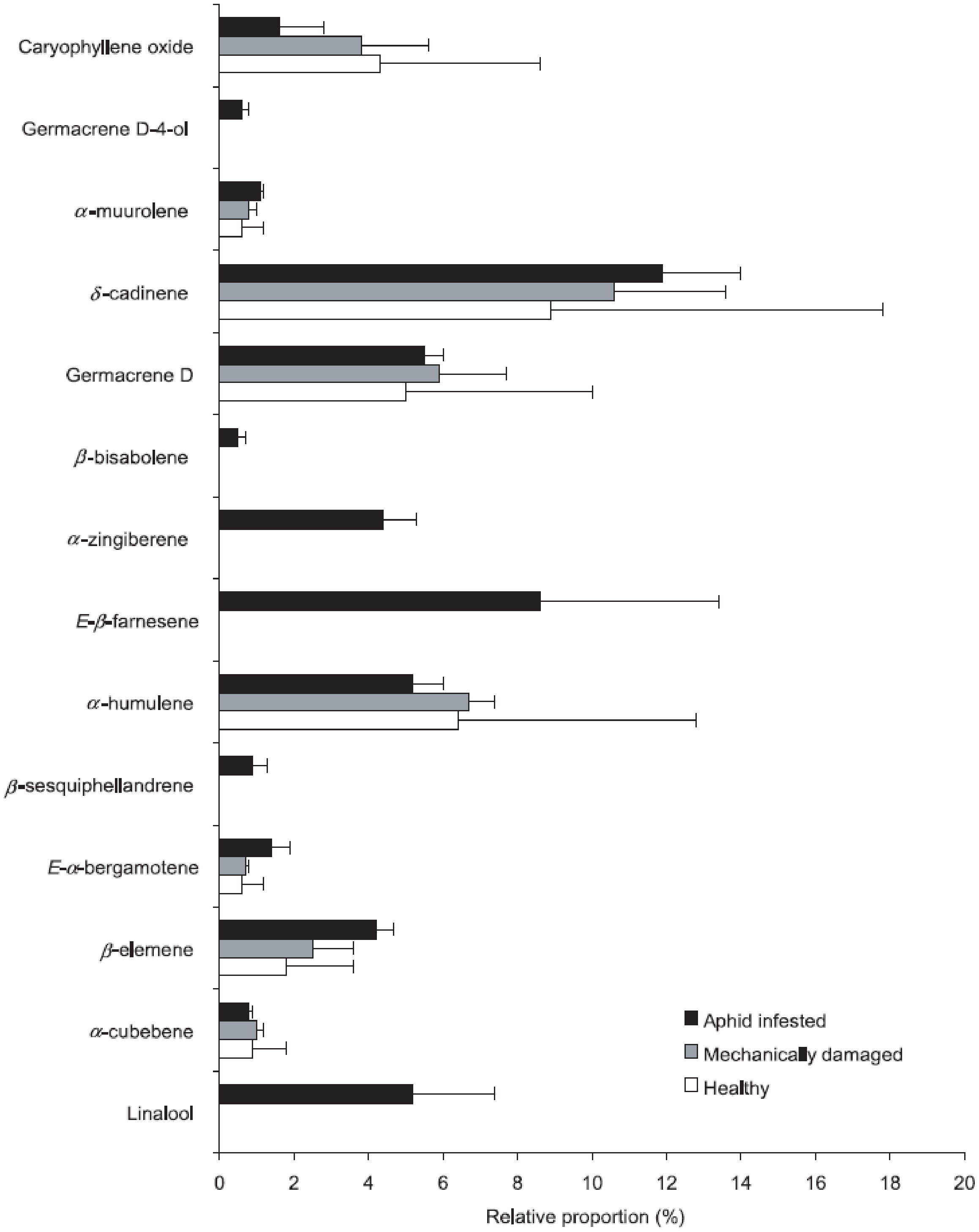

Figure 6. Changes of volatile emission (in relative %) from healthy compared to aphid-infested and mechanically damaged potato host plant. Data points show the mean ± SE of three independent assays.

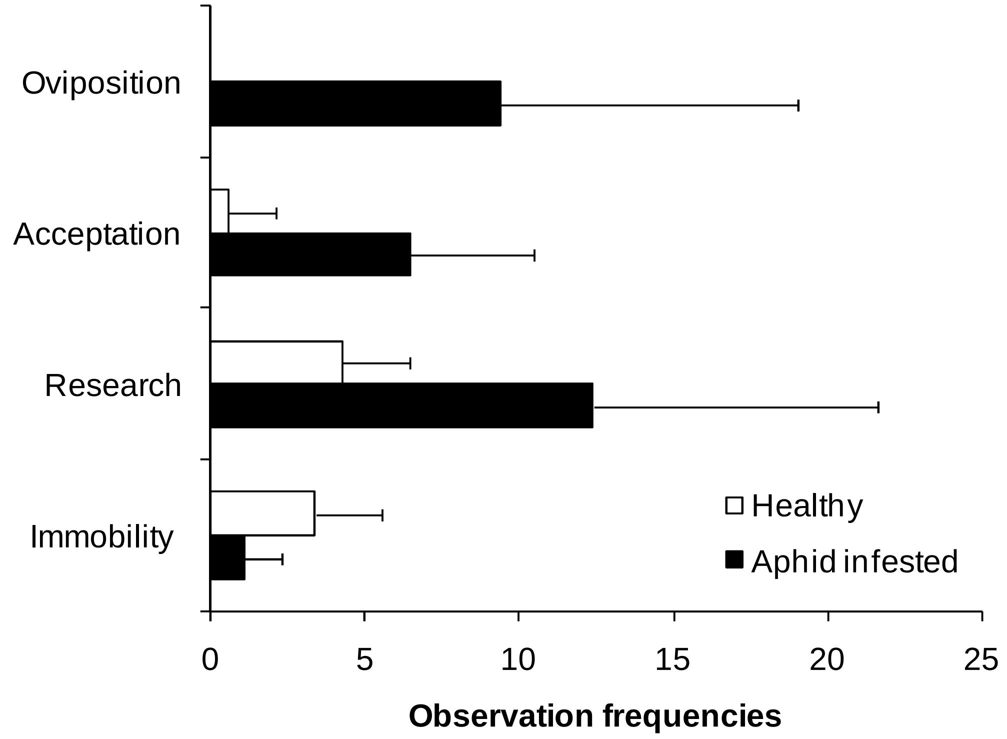

Figure 7. Behavioral sequences (frequencies) of *E. balteatus* females in net cages according to aphid-free or infested host potato plant. Data points show the mean ± SE of 10 independent assays.

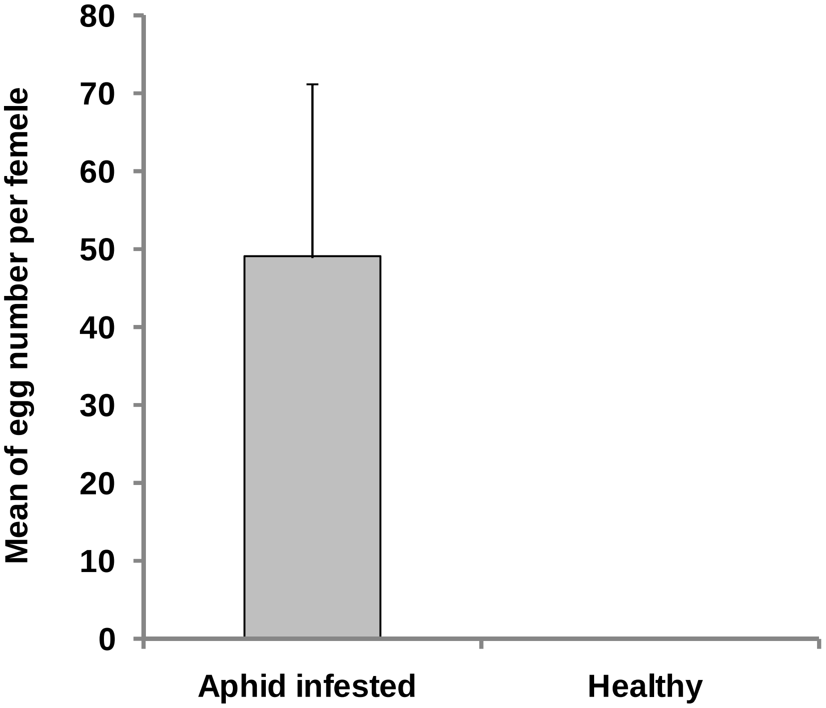

Figure 8. Reproductive efficiency of *E. balteatus* in net cages according to aphid-free or infested potato host plant. Egg numbers were observed after 2 hours of egg-laying.

Discussion

Several tritrophic interaction studies on plant-herbivores natural enemies demonstrated the plant indirect defence mechanisms as an efficient way to cope with pests by the use of volatile infochemicals (Turlings *et al.*, 1990; Turlings & Tumlinson, 1992). When attacked by herbivores, several plant species were shown to emit volatiles that attract natural enemies of the damaging insect pests (Vet & Groenwold, 1990; Vet & Dicke, 1992; Tumlinson *et al.*, 1992). Here we found that the volatile pattern from aphid-infested potatoes was very different from the one from healthy potatoes. Five volatile molecules were emitted by aphid-infested potato plants, while mechanical damage did not have this impact. According to Miles (1999), the salivary proteins injected by the aphid during its feeding on plants seemed to be directly involved in this plant response change when compared to non-aphid damage. In particular, the production of some terpenes was induced by the aphid feeding on plant, including the EβF, a dual active compound towards aphids (as alarm pheromone) and aphidophagous beneficials (i.e. kairomones) (Francis *et al.*, 2004, 2005a, b). Here, the EβF was found to represent 9% of the volatiles related to the *M. persicae* feeding on bean while it did not appear when mechanical damage occurred on plants. Completely different volatile profiles between insect-damaged and mechanically damaged plants were already observed using corn seedlings-beet-armyworm associations. The larvae of the latter pest induced the emission of (*Z*)-3-hexen-1-yl acetate, linalool, (3E)-4,8- dimethyl-1,3,7-nonatriene, indole, 8, *α*-E-bergamotene, EβF, (*E*)-nerolidol, and (3E, 7E)-4,8,12-trimethyl-1,3,7, 11-tridecatetraene when feeding on corn plants. Artificially damaged corn plants led to far fewer volatile releases, in both abundance and diversity (Turlings *et al.*, 1990). The EβF was again one of the particular volatile molecules related to insect-infested plants. For example, the *E. balteatus* aphidophagous predator and females of the *Cotesia marginiventris* (Cresson) parasitic wasp perceived the difference between mechanically and insect-damaged plants to locate armyworm hosts. Also, cucumber plants infested either with thrips, *Frankliniella occidentalis* (Pergande) or spider mites, *Tetranychus urticae* Koch, attracted the predatory bug *Orius laevigatus* (Fieber) (Venzon *et al.*, 1999). In this work, as well as in the previously cited examples, the beneficial decision was based on the odor pattern and volatile abundance from the plant-herbivore combination. The volatile profiles were specific from the first and second level associations in tritrophic interactions, whereas the third level of entomophagous insects benefit from airborne cues from the first two trophic levels (Paré & Tumlinson, 1999). Focusing on a particular group of pests such as aphids, indirect defences from plants leading to the aphidophagous beneficial attraction were observed from aphid-infested plants. For example, *C. septempunctata* responded positively to volatiles from *R. padi* L. aphids infesting barley plants in olfactometer assays (Ninkovic *et al.*, 2001). Francis *et al.* (2004) previously showed that both *Adalia bipunctata* predatory larvae and adults were attracted by EβF emitted from crushed *Acyrthosiphon pisum* Harris and *M. persicae* aphids reared on broad beans (*Vicia faba* L.). In that experiment, EβF, the well-known aphid alarm pheromone, was found to be an effective kairomone for the two-spot ladybird. Using another aphidophagous species, namely *E. balteatus* DeGeer, the infochemical role of aphid-volatile releases, particularly EβF, was also demonstrated (Francis *et al.*, 2005b). Working on another parasitoid, Du *et al.* (1998) identified six volatile compounds (linalool, 6-methyl- 5-hepten-one, *Z*-3-hexen-1-yl acetate, *E*-*β*-ocimene, *Z*- 3-hexen-1-ol and EβF) involved in the attraction of parasitoid *Aphidius ervi* to *A. pisum* infested broad beans. Attacks from phloem-feeding aphids elicit weak responses in contrast to tissue-feeding lepidopteran larvae and mesophyl-sucking insects (Voelckel *et al.*, 2004; De Vos *et al.*, 2005; Zhu-Salzman *et al.*, 2005). This is due to their feeding style: stylet penetrates plant epidermal and parenchymal cells to reach phloem sieves, thereby inflicting minimal wounding to the plant (Miles, 1999). Limited plant damage brings aphids closer to parasites than herbivores. However, we showed that volatile response (terpene emission in particular) of *M. persicae* infested *S. tuberosum* was significantly different from the healthy plants and influenced *E. balteatus* foraging and reproductive behavior. Attraction of natural enemies is known as an indirect defence mechanism and is potent for control of harmful agricultural pests like aphids. Aphidophagous predators are widespread in agro-ecosystem and, among these predators, *E. balteatus* were found to be the most common hoverfly species in crop areas in Belgium (Colignon *et al.*, 2001) and temperate regions (Gilbert, 1986). Once identified, volatile attractants for beneficial insects had to be evaluated in field experiments (James, 2003). Semiochemicals can be used with great success as components of integrated pest management strategies. The way from the discovery to commercial production is long and full of pitfalls but represents an opportunity already illustrated by Birkett and Pickett (2003) for aphid sex pheromones.

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Chapter 5

Aphid colony size discrimination

Aphidophagous hoverflies exploit temporary aphid colonies infesting a wide range of herbaceous plants. Oviposition responses to variation in aphid colony size could reflect adaptations that reduce interspecific competition and optimise the female's searching efforts. Furthermore this behaviour is also thought to be ideal for suppressing aphid populations before they reach damaging levels. Aphid colonies are patchily distributed and they can stay several weeks on their host plant. Syrphid larvae therefore face to a potentially unstable food supply, and hence it may be important to locate aphid infestations quickly. Aphidophagous hoverflies are known to demonstrate a positive density-dependent response to aphid colony size in term of oviposition. They seem to be also able to adjust their oviposition rate according to aphid colony sizes. However, the behavioural mechanisms as how predatory hoverfly females evaluate aphid colony size and adapt their egg-laying accordingly are still unclear.

In the present chapter, a range of experiments were therefore conducted to understand the behavioural mechanisms of the hoverfly E. balteatus in response to different aphid colony sizes. Oviposition behaviour of E. balteatus was first investigated in response to different aphid densities using a leaf-disc system. Secondly, we wanted to identified the volatile organic compounds released in their headspaces from different aphid M. persicae densities. Finaly, the behavioural impacts of the identified chemical coumpounds were also evaluated.

Article 4

Evaluation of hoverfly *Episyrphus balteatus* De Geer (Diptera: Syrphidae) oviposition behaviour toward aphid-infested plants using a leaf disc system

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Abstract – Several aphidophagous beneficials such as parasitoids and predators are known to respond positively to aphid infested plants. The aim of present study was to evaluate the oviposition behaviour of predatory hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphiae) in response to aphid colony size using a leaf-disc bioassay. Three kinds of laboratory experiments were conducted using broad bean plant (*Vicia faba L.*) and aphid *Myzus persicae* Sulzer. In the first experiment, the effect of different densities of aphid *M. persicae* (0, 10 and 100 individuals) on syrphid oviposition response was investigated. Different combinaisons of aphid density/host plants (*V. faba*, *Solanum tuberosum* L., *Solanum nigrum* L.) were Secondly tested toward *E. balteatus* females. In the third experiment, the effect of aphid colony location at different heights (5, 20 and 40cm) on syrphid oviposition response was also studied. A treatment control was run in parallel consisting of *V. faba* leaf-discs without aphids. Our results demonstrated that the number of eggs laid (oviposition rates) by *E. balteatus* female differed significantly in response to increasing aphid colony size infesting leaf-disc system. The means of eggs-laying were: 0.9, 5.3, and 31.2 for 0, 10 and 100 aphid densities respectively. Similar oviposition response was also shown by *E. balteatus* females according to different aphid densities infested different host plants. Aphid colony location at heights of 5 and 20 cm were the most attractive for the hoverfly oviposition. The means of eggs-laying on leaf-discs were: 16.7, 18.5 and 5.8 for 5, 20 and 40cm heights respectively. As result, our leaf-disc system was found to be a practical and efficient way to evaluate *E. balteatus* oviposition behaviour in resoponse to aphid-infested plants under different laboratory conditions.

Key words: *Vicia faba*, *Myzus persicae*, semiochemicals, *Episyrphus balteatus*, leaf disc system, oviposition behaviour.

Reference – Almohamad R, Verheggen FJ, Francis F, Haubruge E. 2006. Evaluation of hoverfly *Episyrphus balteatus* De Geer (Diptera: Syrphidae) oviposition behaviour toward aphid- infested plants using a leaf disc system. *Communications in Agricultural and Applied Biological Sciences, Ghent University*. 71(2 Pt B): 403-412.

Introduction

Predator responses toward variation of different prey densities have been a central theme in ecology theory (Hassell & May, 1974; Kareiva & Odell, 1987) and biological control (Beddington *et al.*, 1978; Murdoch *et al.*, 1985). A predator that responds numerically to increasing pest colony size 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 & Greathead, 1988; Murdoch & Briggs, 1996).

In a tritrophic approach, the semiochemical compounds emitted by aphids or association with their host plants have been found to be attractive for aphid natural enemies (Du *et al.*, 1998; Al Abassi *et al.*, 2000; Ninkovic *et al.*, 2001; Francis *et al.*, 2004). Aphidophagous hoverflies are well-known aphid natural enemies that can have a significant effect in the suppression of aphid populations (Chambers & Adams, 1986; Chambers, 1988). Their foraging and oviposition behaviour were also found to be induced by different volatile chemicals signals realeased from aphids (Budenberge & Powell, 1992; Sutherland *et al.*, 2001; Francis *et al.*, 2005). When forage for a suitable oviposition site, predatory hoverfly females dispose their eggs close to aphid colonies infested plants (Chandler, 1968a; Tenhumberg & Poehling, 1995; Scholz & Poehling, 2000). Previous studies have also shown that there are close relationship between the syrphid ovipostion and the aphid colony size (Dixon, 1959; Volk 1964; Chandler, 1968a; Ito & Iwao, 1977; Geusen-Pfister, 1987; Bargen *et al.*, 1998; Scholz & Poehling, 2000; Sutherland *et al.*, 2001). The study reported here aimed to evaluate the oviposition behaviour of predatory hoverfly *E. balteatus* in response to increating of aphid colony size using a leaf-disc system.

Materials and methods

Plant and insects rearing – Broad bean plants (*Vicia faba* L.) were grown in 30 x 20 x 5 cm plastic trays with a mix of perlite and vermiculite (1/1) and maintained in controlled environment growth rooms at 20 ± 2°C, under a 16:8 Light: Dark photoperiod. Aphid species, namely *Megoura viciae* Buckton and *Myzus persicae* Sulzer were reared on *V. faba*, in separate air-conditioned rooms under the same conditions as above. Adult syrphids were reared in 75 x 60 x 90 cm cages and were provided with bee-collected pollen, sugar and water. *V. faba* plants infested with *M. viciae* were introduced into the cages for 3h every two days to allow oviposition. Hoverfly larvae were mass-reared in aerated plastic boxes (110 x 140 x 40 mm) and were fed daily *ad libitum* with *M. viciae* as standard diet.

Preparation of leaf-disc system –The leaf disc system consisted of (1) a circular piece of *V. faba* leaf, (2) aphids and (3) 7 ml of an agar diet (agar 1% solution w:w) prepared in a small petri dish (25 mm diameter) to reduce desiccation. The aphids infested leaf-disc was kept for 24h in a controlled condition incubator (16:8 Light: Dark; 20 ± 1°C) before testing. The leaf-disc system was then positioned on a Plexiglas holder 20 cm high before being exposed to a *E. balteatus* females in a net cage (30 x 30 x 60 cm) (Figure 9.A).

Oviposition responses of hoverfly *E. balteatus*

Three experiments were conducted to evaluate syrphid oviposition in response to aphid *M. persicae* colony size using leaf-disc system under different conditions:

Experiment 1.Impact of different aphid colony sizes

In third-choice experiment, three leaf-discs previously described, infested with three densities of aphid *M. persicae* (0, 10 and 100 individuals) were placed on three Plexiglas holders at height of 20cm in net cage (30 x 30 x 60 cm). A single *E. balteatus* female was then introduced into the net cage in presence of three leaf-discs infested with three aphid densities and allowed to lay eggs for 3h. The eggs laid on each leaf-disc were then counted. Experiments were conducted in an air-conditioned room at 21 ± 1 °C. Ten replicates were performed for this experiment. In similar way that described above under the same conditions, this experiment was also repeated again using broad bean plants in order to evaluate the oviposition response of predatory hoverfly with leaf-disc system compared to the whole *V. faba* plants. Wheres three *V. faba* plants (stem with 6 true leaves, 20 cm high) infested with the same aphid densities (0, 10 and 100)

Experiment 2. Impact of different combinaisons of aphid density/host plants

Aphids *M. persicae* were reared on broad bean plants *V. faba* and two solanaceous plant species, *Solanum tuberosum* L. “Binch” variety and *Solanum nigrum* L. (Solanaceae) growing in 30 x 20 x 5 cm plastic trays filed with a mix of perlit and vermiculte (1:1) in separate controlled rooms (L16:D8 and 20 ± 1). Three leaf-discs infested with three aphid densities (25, 75 and 125 individuals) were prepared fom each combinaison *M. persicae*/host plant. 9 Gravide *E. balteatus* females were transferred into separate cages. Each aphid density/leaf host plant-disc was then offered to each syrphid female on on Plexiglas holders at height 20 cm. Each two days, aphid densité infested leaf host plant-discs were presented in a randomised sequence (i.e, a no-choice situation, with only one aphid density available at any time) to each syrphid female. Each presentation of 3 different densities of *M. persicae*/ host plant lasted for 3h. The eggs laid by syrphif felames were then counted on each aphid density/leaf disc. The aphids were replaced with a new excess of aphid *M. persicae* for each presentation. Every two days, females had the choice to oviposit on the three different aphid densities of each host plant in net cage (30 x 30 x 60 cm). Experiments were conducted in a controlled environment room at 21±1 C°, under a L16: D8 photoperiod. This experiment was replicated 6 times for each aphid density-leaf host plant.

Experiment 3. Impact of aphid colony location

To assess the effect of aphid colony location on syrphid oviposition response, three *V. faba* leaf-discs were infested with constant density of *M. persicae* (125 individuals), and then placed on different heights of plexiglass holders (5, 20 and 40 cm) in a net cage. In third choice experiment, a single *E. balteatus female* was introduced into a net cage in presence of three treatments and allowed to lay eggs for 3h. The eggs laid were then counted on each aphid-leaf disc. Experiments were conducted in similar conditions as described above. Tested *E. balteatus* females were approximately 20-30 days old and no induction of oviposition had been realised for 24h. This experiment was replicated 10 times (Figure 9B).

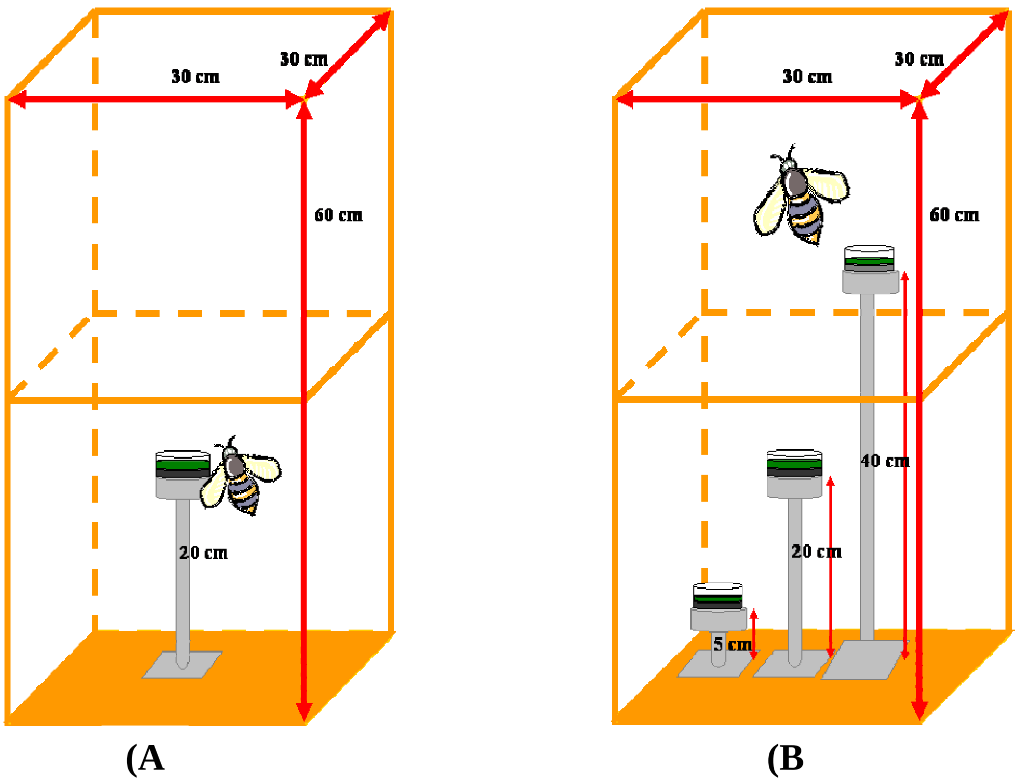

Figure 9. Illustration of leaf disc system used to assess the fecundity behaviour of *E. balteatus* toward aphid colony size on single height (A), and location on different heights (B).

Statistical analysis

All mean comparison tests were realised using using Minitab® software (12.2 version, Minitab Inc, State College, PA, USA). Tukey's test was used to compar the mean numbers of eggs laid by females on aphid-leaf disc system under different conditions. Data on number of eggs laid by hoverfly female were normalised before statistical analysis.

Results

Experiment 1. Impact of different aphid colony sizes

Our results demonstrated that aphid colony size infested leaf-disc system influence significantly on *E. balteatus* oviposition rates (Figure 10A). Where, females significantly laid more eggs on leaf-discs infested with high aphid density (100) than those with free and low aphid density infestation (0 and 10 individuals) (Tukey' test, $t = 14.32$, $P < 0.001$; $t = 8.92$, $P < 0.001$ respectively). Similar oviposition behaviour was also shown by *E. balteatus* females in response to increasing aphid colony size infested whole *V. faba* plants (Figure 10B).

Experiment 2. Impact of different combinaisons of aphid density/host plant

The number of eggs laid by *E. balteatus* female increased significantly in response to increasing aphid colony size infested different host plants using leaf disc system (Figure 11). Fewer eggs were laid on leaf-disc infested with small aphid colony (25 individuals) compared to those infested with large aphid colonies (75 and 125 individuals) ($P < 0.05$).

Experiment 3. Impact of aphid colony location

Results of third–choice experiment demonstrated that aphid colony located at different heights had significant effect on oviposition response of predatory hoverfly female (Figure 12). 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 (Tukey' test, $t = 2.69$, $P = 0.03$; $t = 3.30$, $P = 0.007$ respectively) (Figure 12).

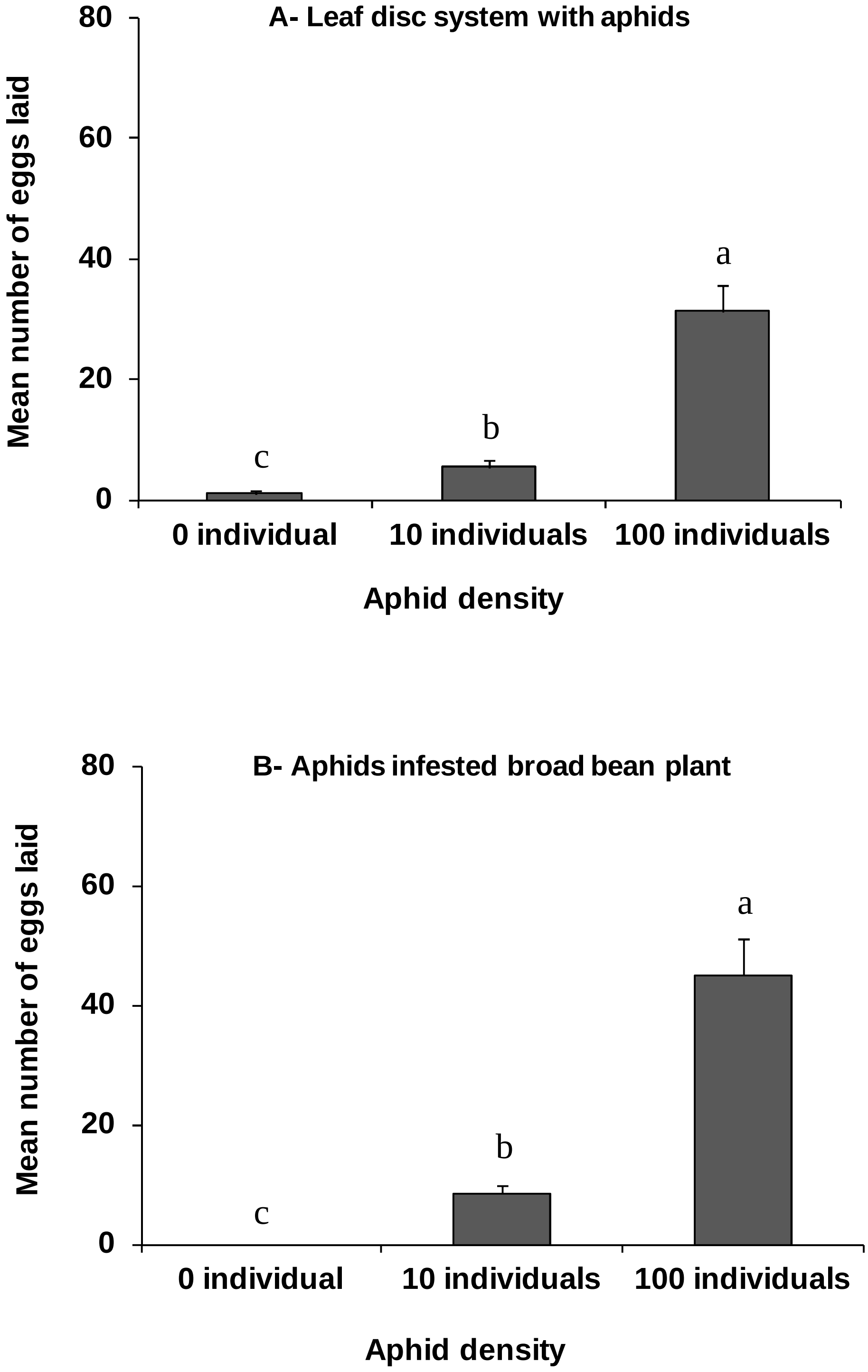

Figure 10. Mean number of *E. balteatus* eggs laid on leaf disc system (A) and whole broad bean plants (B) in response to different densities of *M. persicae*. Means (± SE) marked with different letters indicate significant difference between treatments (Tukey' test, $P < 0.05$).

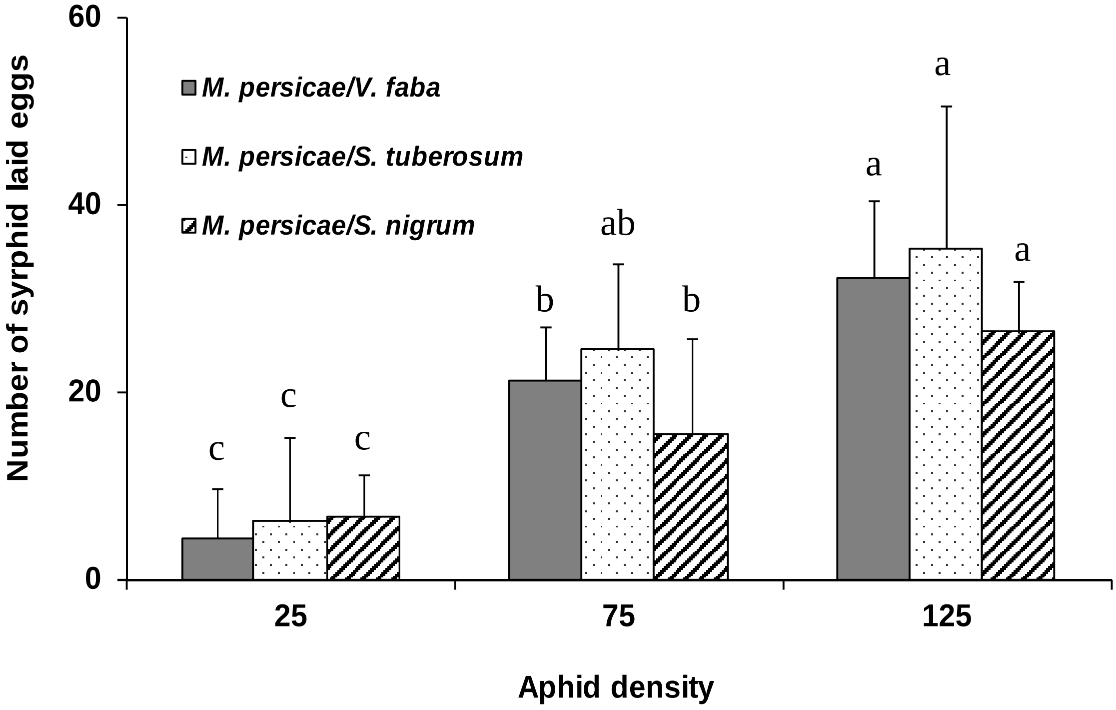

Figure 11. Mean numbers of *E. balteatus* eggs laid on host plants infested with different densities of *M. persicae* colony size. Different letters indicate significant differences between treatments (P < 0.05). Bars indicate standard errors of the means.

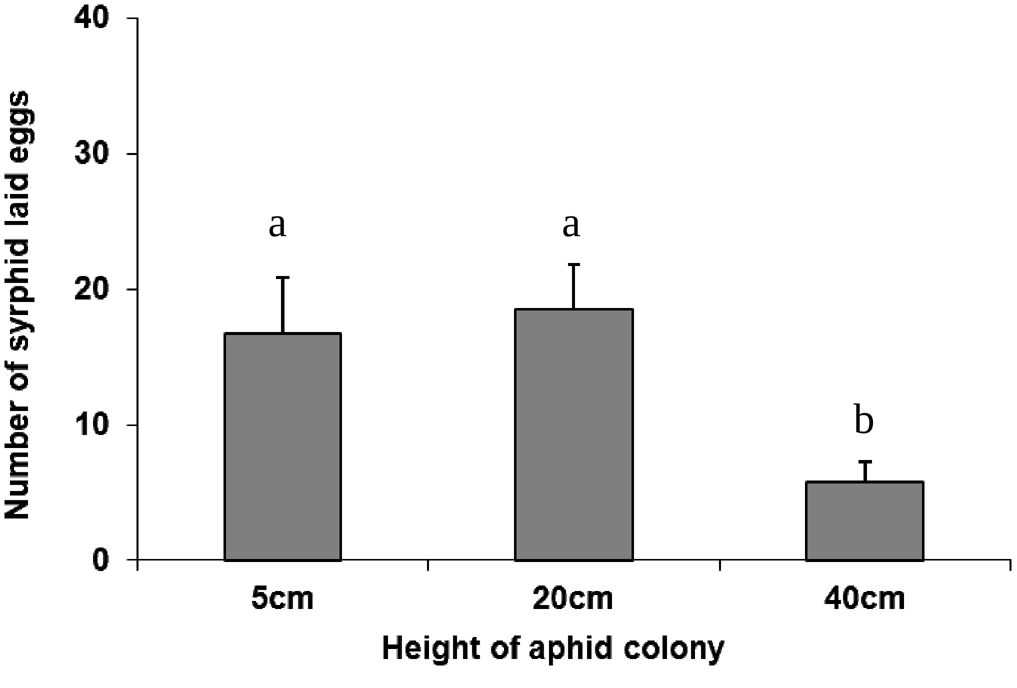

Figure 12. Effect of *M. persicae* aphid colony location on *E. balteatus* oviposition rates. Means (± SE) marked with different letters indicate significant difference between treatments (Tukey's test, P < 0.05).

Discussion

Field and laboratory observations have previously demonstrated that the oviposition rate of aphidophagous syrphid flies was found to be positively correlated with increasing of aphid colony size (Dixon, 1959; Ito & Iwao., 1977; Geusen-Pfister, 1987; Tenhumberg, 1993; Bargen *et al.*, 1998; Scholz & Poehling, 2000; Sutherland *et al.*, 2001). Our experiments demonstrated that *E. balteatus* female responded positively to increasing of aphid colony size on leaf-disc system in terms of oviposition rates. Similar oviposition response was also exhibited by syrphid female according to different aphid colony sizes infested broad bean plants. As result, the leaf-disc system could be considered as good method to evaluate syrphid oviposition behaviour.

It has been also demonstrated that the suitability of an oviposition site does not only depend on the number of aphids present at the time of oviposition, but it may depend on the quality of the aphid colony (Kan & Sasakawa, 1986, Kan, 1988a,b). It is possible that females avoid very high aphid infestation, since they are subjected to increased migration of the prey (Kan, 1988a,b), and therefore may not support the full development of several syrphid larvae. Other aphid colony factors may be involving in syrphid oviposition responses (e.g. chemical cues released from aphid associated with host plant) (Dixon, 1959; Bombosch & Volk, 1966). In present study, we found that host plant infested with different aphid colony sizes had significant effect on *E. balteatus* oviposition behaviour.

The height preference of various syrphid species has been shown to be relative with their habitat preferences (Chandler, 1968b). The latter author demonstrated that univoltine syrphid species that develop in spring, they tended to oviposit at height around 180 cm because aphids are present on trees and shrubs but are rare on herbaceous plants, while all syrphids species that develop in early summer, they tended to oviposit at height 30 cm because aphids are abundant on herbaceous cover. Those spcies 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, leaf-disc system using in present laboratory experiments could be considered as a useful and efficient method to evaluate *E. balteatus* oviposition behaviour in response to aphid infested plants.

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Article 5

Emission of alarm pheromone by non-preyed aphid colonies

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Abstract – The sesquiterpene (E)-β-Farnesene (EβF) is the primary component of the alarm pheromone of most aphid species. It is released in response to physical stress including attack by natural enemies and causes aphids to cease feeding and disperse. EβF also acts as a kairomonal cue for aphid natural enemies. In this study, we collected the headspace volatiles released by aphid colonies of different sizes. Gas chromatography-mass spectrometry analysis demonstrated the presence of EβF in the absence of predator attack. A quadratic relationship was found between the released (*E*)-β-farnesene amounts and aphid colony size. Behavioural impact of aphid alarm pheromone toward *Episyrphus balteatus* female oviposition behaviour was also demonstrated in this work. These results highlight the primary role of the small but continuous release of aphid alarm pheromone in mechanisms of decision-making by aphid predators during their foraging and egg-laying behaviour.

Key words: Semiochemicals, *Episyrphus balteatus*, *Myzus persicae*, aphid alarm pheromone, (*E*)-β-farnesene.

Reference – Almohamad R., Verheggen FJ., Francis F., Lognay G. & Haubruge E. (2008). Emission of alarm pheromone by non-preyed aphid colonies. *Journal of Applied Entomology*. 132 (8): 601-604.

Introduction

(E)-β-Farnesene (EβF), the main component of the aphid alarm pheromone was identified in 16 aphid species, alone or associated with other molecules (Francis et al. 2005). Previous behavioural studies have demonstrated the kairomonal role of EβF in various aphid natural enemies, including ladybeetles, hoverflies and parasitic hymenoptera (Du et al. 1998; Al Abassi et al. 2000; Harmel et al. 2007; Verheggen et al. 2007, 2008).

In the hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphicae), there is a good evidence from laboratory and field studies for the existence of a positive density-dependent response to aphid colony size in term of oviposition (Dixon 1959; Chandler 1968; Itô and Iwao 1977; Bargen et al. 1998; Scholz and Poehling 2000; Sutherland et al. 2001; Almohamad et al. 2006). However, there is only little work on the role of odour cues in predatory hoverflies attraction (Laubertie et al. 2006; Almohamad et al. 2007, 2008; Verheggen et al. 2008).

This study aims at understanding the role of chemical cues released from non-preyed aphid colonies on decision–making processes that lead to oviposition in female *E. balteatus*. In order to evaluate the olfactory signal released by the tested colonies, we quantified the volatile organic compounds released in their headspaces. The oviposition behaviour of *E. balteatus* was subsequently investigated with respect to *Myzus persicae* Sulzer colonies of different sizes.

Materials and Methods

Plants and insects – Broad bean plants (*Vicia faba* L.) were grown in 30 x 20 x 5 cm plastic trays filled with a mix of perlite and vermiculite (1/1) and maintained in controlled environment growth rooms (16:8 Light: Dark ; 20 ± 1°C). Two aphid species, namely *M. persicae* and *Megoura viciae* Buckton were taken from stock rearing on *V. faba*, in separate air-conditioned rooms under the same conditions as above. Adult *E. balteatus* were reared in 75 x 60 x 90 cm cages and were provided with bee-collected pollen, sugar and water. Broad beans infested with *M. viciae* were introduced into the cages for 3 h every two days to allow oviposition. Hoverfly larvae were mass-reared in aerated plastic boxes (110 x 140 x 40 mm) and were fed daily *ad libitum* with *M. viciae* as standard diet.

Leaf disc system – The leaf disc-system consisted of (1) a circular piece of *V. faba* leaf, (2) aphids and (3) 7 ml of an agar diet (agar 1% solution w:w), placed in a 25 mm diameter Petri dish, to reduce desiccation. Leaves were infested with different quantities of *M. persicae* and were kept for 24h in a controlled conditions incubator (16:8 Light: Dark; 20 ± 1°C) before testing.

Influence of aphid colony size on syrphid oviposition rate – In no-choice experiments, a single *E. balteatus* female was introduced in a net cage and allowed to lay eggs for 3h on the leaf disc supporting a *M. persicae* colony made of 25, 75 or 125 individuals. The leaf disc system was placed on a Plexiglass holder at a height of 20 cm. This setup was previously shown as an efficient method to evaluate the oviposition behaviour of the hoverfly *E. balteatus* in response to aphid-infested plants (Almohamad et al. 2006). The number of eggs laid (oviposition rate) on the leaf disc was counted. This experiment was replicated 10 times for each aphid colony size.

Collection and analysis of volatile chemical emissions

Volatile collection system – Volatile chemicals were collected using a push/pull volatile collection system consisting of a glass air-collection chamber (Schott®, 12 cm base-diameter, 35 cm high) (Schott, Mainz, Germany) placed inside an incubator set at 21 ± 1°C, and previously washed with hot water and *n*-hexane. The leaf disc system was placed on a Plexiglass holder similar to those used in the above-mentioned bio-assays. Incoming air was pushed through an in-line activated charcoal filter before entering the glass chamber at a flow of 200 ml/min. The volatile-enriched air was then pulled through an adsorption trap containing 40 mg SuperQ® (Alltech, Deerfield, IL, USA). Six replicates were conducted for each aphid colony size and four replicates for the control (i.e. an aphid-free leaf disc system). Volatiles were collected during 3h. Filters were eluted with 150 µl of *n*-hexane and nonyl acetate (400 ng) was added to each sample as internal standard. The extracted samples were directly stored in a freezer at − 80 °C until GC analysis. Identification and quantification were performed respectively by GC-mass Spectrometry (MS) and GC- Flame Ionization Detector (FID).

Statistical analysis

Regression analysis was used to correlate aphid density with number of eggs laid by female *E. balteatus* and amounts of released EβF. All analysis were performed using Minitab® software (14.2 version, Minitab Inc, State College, PA, USA).

Results and Discussion

EβF emission from non-preyed *M. persicae* was demonstrated in the present study by volatile collection and subsequent GC-MS analysis. Regression analysis revealed that there was a significant quadratic relationship between the amount of released EβF (*Y*) and *M. persicae* colony sizes (*X*) ($F_{2,23} = 14.89$; $P < 0.001$; $r^2 = 0.9997$). This relationship can be represented as $Y = 0.0143X^2 - 0.2265X$ (Figure 13).

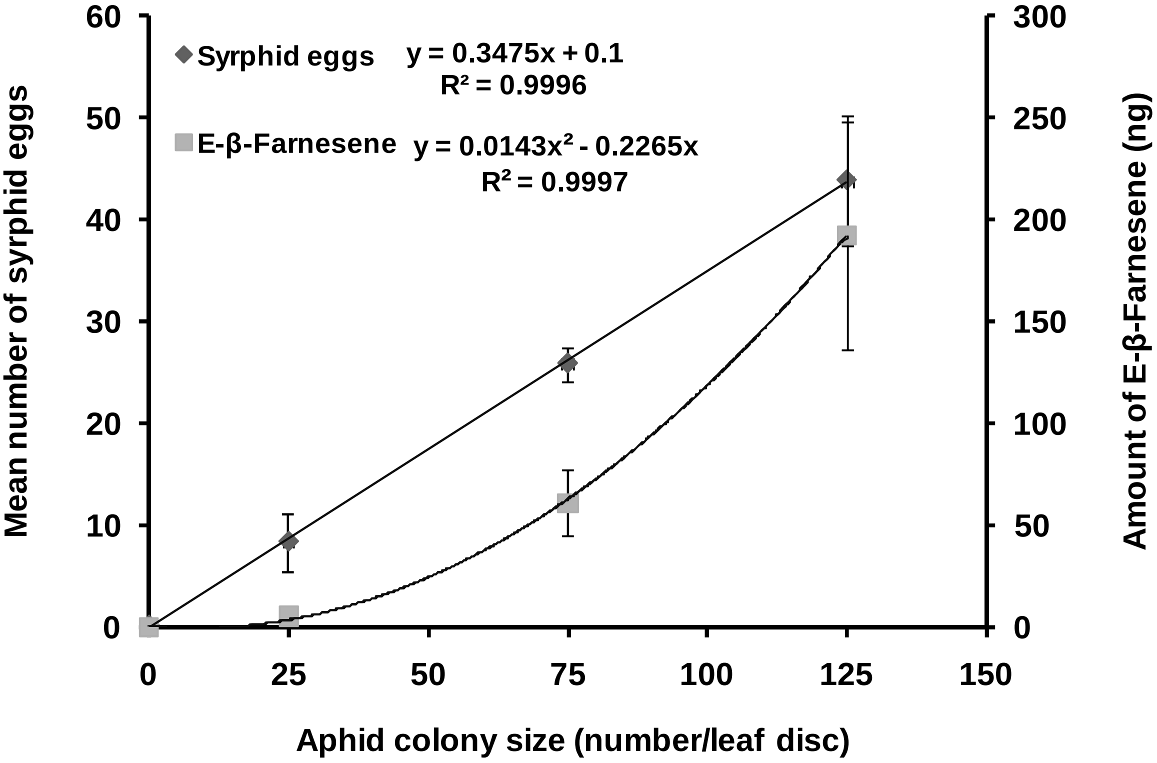

Figure 13. Amount of (E)-β-farnesene and mean number of *Episyrphus balteatus* eggs laid in response to increasing *Myzus persicae* colony size on bean leaf disc. Bars indicate standard errors of the means.

Other chemical compounds were also identified, such as hexanal, 3-methyl-2-pentanone, β-terpinene, 6-methyl-5-hepten-2-one and limonene. These latter compounds were found to be released from leaf-disc system and they were not specifically induced by the presence of *M. persicae* colony on leaf disc. Geranyl acetone was also identified and its amount slightly decreased with increasing aphid colony size.

The constant emission of EβF in absence of predators can have both positive and negative effects. Aphids may have advantage by releasing small amounts of alarm pheromone in the case of crowded colonies, thereby causing dispersion of the individuals and help preserving the host plant. This effect would complete the already well-known effect of winged-morph induction described by Kunert et al (2005). However, a constant emission of alarm pheromone increases the risk of the colony being located by a natural enemy.

According to previous observations, nearly no egg was laid in absence of aphids on the leaf disc system (Figure 13). The number of eggs laid (*Y*) increased significantly and linearly with the size of the aphid colony (*X*) ($F_{1,39} = 94.12$; $P < 0.001$; $r^2 = 0.9996$), according to $Y = 0.3475X + 0.1$. Several studies suggested that the oviposition rate of syrphid females was a function of aphid densities (Chandler 1968; Itô and Iwao 1977; Bargen et al. 1998; Scholz and Poehling 2000; Sutherland et al. 2001; Almohamad et al. 2006). Our regression analysis suggests an adaptive oviposition behaviour leading the emerging larvae to locate immediately sufficient food resources. According to Bargen et al. (1998), the number of eggs laid by female hoverflies does not only depend on aphid quantity on the plant, but also on the presence and quantity of oviposition-eliciting substances emitted from the prey and the infested plant. Previous observations have indeed shown that predatory hoverflies oviposit in response to volatile compounds emitted from aphids and their liquid secretions such as honeydew (Dixon 1959; Bombosch and Volk 1966; Budenberg and Powell 1992; Shonouda et al. 1998; Verheggen et al. 2008).

Along with the previous results of Verheggen et al. (2008) on the role of synthetic EβF as oviposition stimulant, these results demonstrate the strong involvement of aphid alarm pheromone in aphidophagous syrphid oviposition behaviour.

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Article 6

Impact of aphid colony size and associated induced plant volatiles on searching and oviposition behaviour of a predatory hoverfly

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Abstract – Volatile chemicals emitted by aphids or aphid-infested plants act as kairomonal substances for several aphid natural enemies, and are therefore considered as indirect defense for the infested plants. In the present study, the foraging and oviposition behaviour of the aphid specific predator, *Episyrphus balteatus* DeGeer (Diptera: Syrphidae), was investigated with respect to the aphid colony size, using a leaf disc bioassay. Female *E. balteatus* exhibited pronounced searching and acceptance behaviour, leading to egg laying, in response to large *Myzus persicae* Sulzer (Homoptera: Aphididae) colony sizes. Behavioural impacts of synthetic aphid alarm pheromone and geranyl acetone toward *E. balteatus* female foraging and oviposition behaviour were also demonstrated in this work. These results highlight the role of aphid semiochemicals in predatory hoverfly attraction and provided an opportunity to elucidate some mechanisms of decision-making by female syrphid predators during their foraging and egg-laying behaviour.

Key words: *Episyrphus balteatus*, foraging behaviour, *M. periscae*, (*E*)-β-farnesene, geranyl acetone.

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Introduction

Volatile chemical signals released by herbivore-infested plants serve as olfactory cues for parasitoids (Du *et al*., 1998; De Moraes *et al*., 1998; Van Loon *et al*., 2000) and predators (Evans & Dixon, 1986; Dicke, 1999; Ninkovic *et al*., 2001). According to Chandler (1968a), aphidophagous syrphid species have been divided into two groups: (1) phytozetic syrphid species, such as *Melanostoma mellinum* (L) (Diptera: Syrphidae), that rely more on plant–derived stimuli than on aphid stimuli, and (2) aphidozetic species, such as *Episyrphus balteatus*, that use aphid-derived chemicals to locate their prey and subsequent oviposition sites. Several studies have demonstrated that specific blend of odours produced by injured plants or released by the pests are attractive to certain predators and parasitoids (Vet & Dicke, 1992 Dicke, 1994). It is likely that aphid-associated odours are interpreted by female *E. balteatus* as olfactory cues orientating them toward aphid-infested sites (Shonouda *et al*., 1998). Honeydew, for example, acts as an oviposition stimulant for syrphid females and as an olfactory cue used in the location of aphid colonies (Budenberg & Powell, 1992; Bargen *et al*., 1998; Sutherland *et al*., 2001). (E)-β-Farnesene (EβF), the main component of the aphid alarm pheromone was identified in 16 aphid species, alone or associated with other molecules (Francis *et al*., 2005a). Previous behavioural studies have demonstrated the kairomonal role of EβF in various aphid natural enemies, including ladybeetles, hoverflies and parasitic hymenoptera (Du *et al*., 1998; Al Abassi *et al*., 2000; Francis *et al*., 2004; Harmel *et al*., 2007; Verheggen *et al*., 2007, 2008). (E)-β-Farnesene, associated with other natural molecules such as α-pinene, β-pinene and limonene, has also been used efficiently as reliable olfactory cues by syrphid larvae in prey location (Francis *et al*., 2005b).

Because of its high reproductive rate, voracity and degree of adaptation, *E. balteatus* is considered as an efficient aphid natural enemy (Chambers, 1988). Moreover, its high mobility, enabling it to lay eggs over large areas (Schneider, 1984; Chambers, 1988, 1991), and to locate aphid colonies earlier in the season than other aphidophagous predators (Hagen & Van den Bosch, 1968; Horn, 1981; Dixon, 2000). Previous studies have demonstrated that the oviposition rate of hoverfly *E. balteatus* females was a function of aphid densities (Chandler, 1968 b; Itô & Iwao, 1977; Geusen-Pfister, 1987; Bargen *et al*., 1998; Scholz & Poehling, 2000; Sutherland *et al.*, 2001; Almohamad *et al*., 2006). However, behavioural responses of predatory hoverflies to odour cues induced from aphid colony size have received little attention (Laubertie *et al*., 2006; Almohamad *et al*., 2008a; Verheggen *et al*., 2008).

In the previous study of Almohamad *et al*. (2008b), there was a significant quadratic relationship between the amount of released EβF and *M. persicae* colony sizes. Geranyl acetone was also identified and its amount slightly decreased with increasing aphid colony size. The present study aims to assess the foraging and oviposition behaviour of *E. balteatus* females with respect to different densities of *M. persicae* colonies. The behavioural impacts of the substances volatiles ((E)-β-farnesene and geranyl acetone) were also evaluated toward females of predatory hoverfly.

Materials and Methods

Plants and insects - Broad bean plants (*Vicia faba* L.) were grown in 30 x 20 x 5 cm plastic trays filled with a mix of perlite and vermiculite (1/1) and maintained in controlled environment growth rooms (16:8 Light: Dark ; 20 ± 1°C). Two aphid species, namely *M. persicae* and *Megoura viciae* Buckton were taken from stock rearing on *V. faba*, in separate air-conditioned rooms under the same conditions as above. Adult *E. balteatus* were reared in 75 x 60 x 90 cm cages and were provided with bee-collected pollen, sugar and water. Broad beans infested with *M. viciae* were introduced into the cages for 3 h every two days to allow oviposition. Hoverfly larvae were mass-reared in aerated plastic boxes (110 x 140 x 40 mm) and were fed daily *ad libitum* with *M. viciae* as standard diet.

Leaf disc system – The leaf disc-system consisted of (1) a circular piece of *V. faba* leaf, (2) aphids and (3) 7 ml of an agar diet (agar 1% solution w:w), placed in a 25 mm diameter Petri dish, to reduce desiccation. Leaves were infested with different quantities of *M. persicae* and were kept for 24h in a controlled conditions incubator (16:8 Light: Dark; 20 ± 1°C) before testing. The leaf disc system was placed on a Plexiglass holder at a height of 20 cm before being exposed to a hoverfly predator. This setup was previously shown as an efficient method to evaluate the oviposition behaviour of the hoverfly *E. balteatus* in response to aphid-infested plants (Almohamad *et al*., 2006).

Influence of aphid colony size on syrphid behaviour – In no-choice experiments, a single *E. balteatus* female was placed in a net cage (30x30x60 cm) in presence of the leaf disc system previously described and supporting a *M. persicae* colony made of 25, 75 or 125 individuals. A non-infested leaf was used as a control. Their foraging behaviour was recorded for 10 minutes using the Observer® (Noldus Information Technology, version 5.0, Wageningen, The Netherland). The four behavioural events that were observed are grouped as described below:

1. Immobility: when the hoverfly stayed on the net cage without moving;

2. Searching: when the syrphid flew in the cage around the infested plant, either in an extensive or intensive way;

3. Acceptation: when the hoverfly landed on the plant, stayed immobile or walked on it, made proboscis extension on the plant surface;

4. Oviposition: when the syrphid had abdomen-bending and laid eggs.

Behavioural observations were conducted in an air-conditioned room at 21 ± 1°C. Hoverfly females were approximately 20-30 days old and no oviposition had occurred for 24h prior the experiment. This experiment was repeated 10 times for each treatment, including the control and three aphid densities.

Impact of (E)-β-farnesene and geranyl acetone on syrphid behaviour – We assessed EβF and geranyl acetone infochemical role toward *E. balteatus* female behaviour by placing 100 µl of a 400 ng/µl solution of EβF or geranyl acetone (diluted in paraffin oil) in a rubber septum placed itself on a leaf disc system without aphids, on the top of a Plexiglass holder. A treatment control was also run in parallel and consisted of leaf with only a rubber septum with only paraffin oil. A single hoverfly female was introduced in the cage and its behaviour was observed for 10 min. Ten replicates were performed for each treatment.

Statistical analysis

Behavioural sequences in response to different aphid colony sizes were compared using Tukey's test, and Dunnett's test was also performed to compare behavioural responses to tested chemical compounds. All analysis were performed using Minitab® software (14.2 version, Minitab Inc, State College, PA, USA).

Results

Influence of aphid colony size on syrphid behaviour – The mean frequencies and mean durations observed for each *E. balteatus* behavioural event are presented in (Figure 14-A, B). Higher aphid densities lead to an increase of searching ($F_{3,39}$ = 3.94; P = 0.019), acceptance ($F_{3,39}$ = 20.95; P < 0.001) and oviposition behaviours ($F_{3,39}$ = 24.95; P < 0.001) in terms of frequencies (Figure 14-A). In addition, *E. balteatus* females did not exhibit abdominal protractions or egg-laying behaviour in absence of aphids.

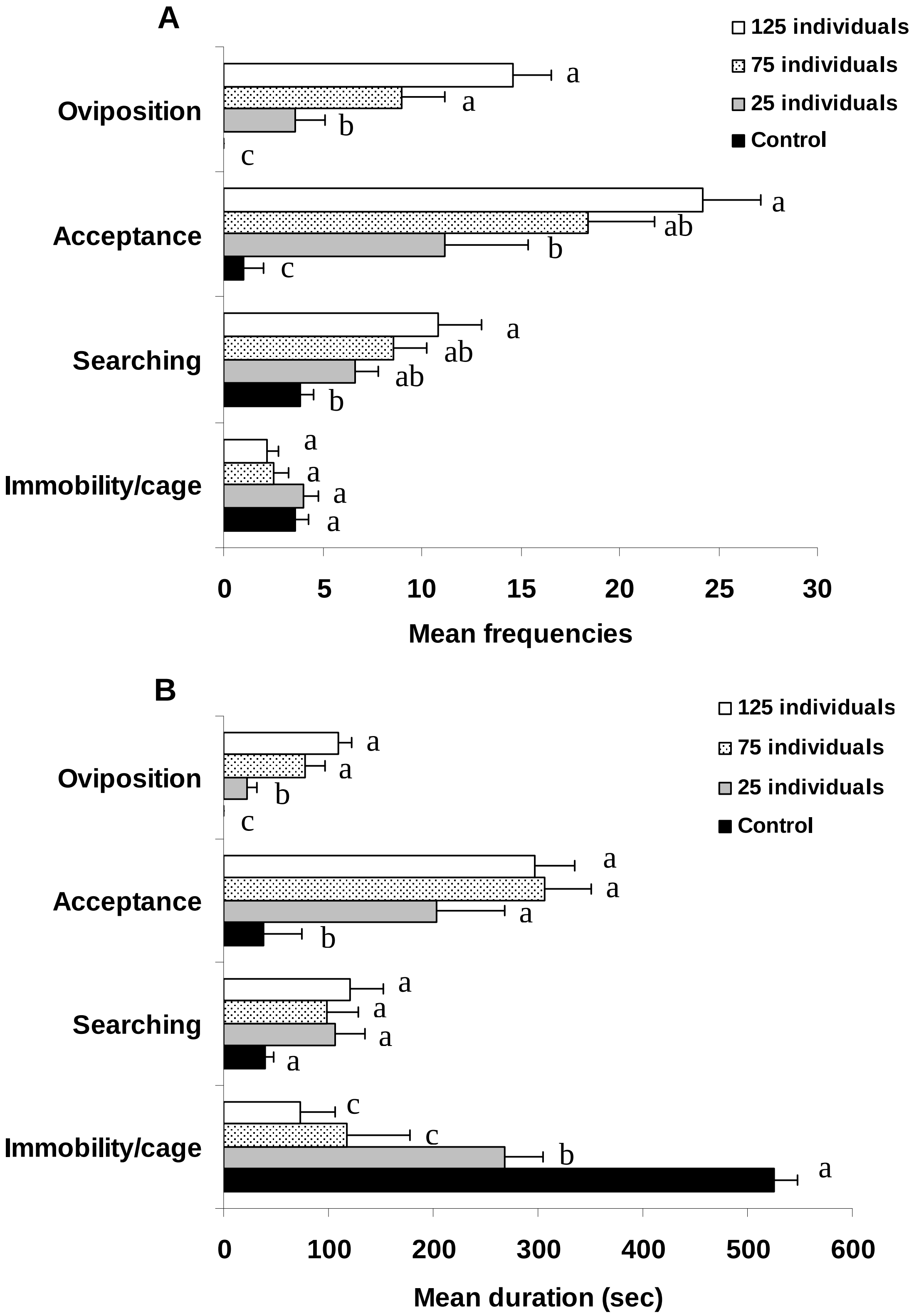

Figure 14. Mean frequencies and durations (± SE) of behavioural observations of *E. balteatus* females in response to increasing aphid *M. persicae* colony size on leaf disc in no-choice experiment. Different letters above bars indicate significant differences between treatments (ANOVA, Tukey's test, $P < 0.05$).

While the hoverfly predator increases its mobility in presence of large aphid colonies, the duration allowed for searching was not affected ($F_{3,39}$ = 1.96; P = 0.193). Time tacken for acceptance and oviposition behaviours were however increased with larger aphid colonies ($F_{3,39}$ = 16.98; P < 0.001 and $F_{3,39}$ = 30.03; P < 0.001, respectively).

***Influence of (E)-β-farnesene and geranyl acetone on syrphid behaviour* –** Compared to the control, EβF induced higher frequencies of *E. balteatus* female searching (t_{obs} = 3.49; P = 0.003) and acceptance behaviour (t_{obs} = 3.44; P = 0.004), while the behavioural responses of *E. balteatus* female was not affected by the presence of geranyl acetone in term searching (t_{obs} = -1.26; P = 0.358) and acceptance behaviour (t_{obs} = -0.25; P = 0.957) (Figure 15-A). Hoverfly female also exhibited abdominal protraction and egg-laying behaviour in response to EβF, while this behaviour was not observed in presence of geranyl acetone.

In presence of EβF, females *E. balteatus* spent more time searching (t = 2.98; P = 0.012) and accepting the host plant (t = 2.73; P = 0.021). The time spent for searching (t_{obs} = - 0.53; *P* = 0.821) and accepting the host plant (t_{obs} = - 0.08; P = 0.995) did not significantly change in presence of geranyl acetone when compared to the control (Figure 15-B). *Episyrphus balteatus* was more mobile in response to the odour of EβF (t = - 4.55; P < 0.001). As a result, (*E*)-β-farnesene demonstrated its effective kairomonal role on *E. balteatus* female foraging behaviour and acted as an oviposition stimulant.

Discussion and conclusions

Our experiments demonstrated that the foraging and oviposition behaviour of individual *E. balteatus* females was dependent of prey colony size. In previous experiments of Sutherland *et al*. (2001), hoverfly females failed to exhibit enhanced behavioural responses, in terms of approaches and landings, to artificial leaves with the highest numbers of aphids. Our results show that *E. balteatus* females exhibited pronounced searching, acceptance (landing, walking, and proboscis extension) and abdominal protraction leading to egg-laying behaviour in response to increasing aphid colony size. These differences of behavioural responses may be due to the dispersion way of individual aphids on the leaf disc. In our experiments, females are allowed to forage in an optimal way as they could access all oviposition cues, including visual, olfactory and gustatory stimuli (i.e. aphid-, and leaf-produced volatiles), in opposition to the study of Sutherland *et al*. (2001), where females were unable to see the colony after landing because individual aphids were contained in clip-cages with a bean leaf.

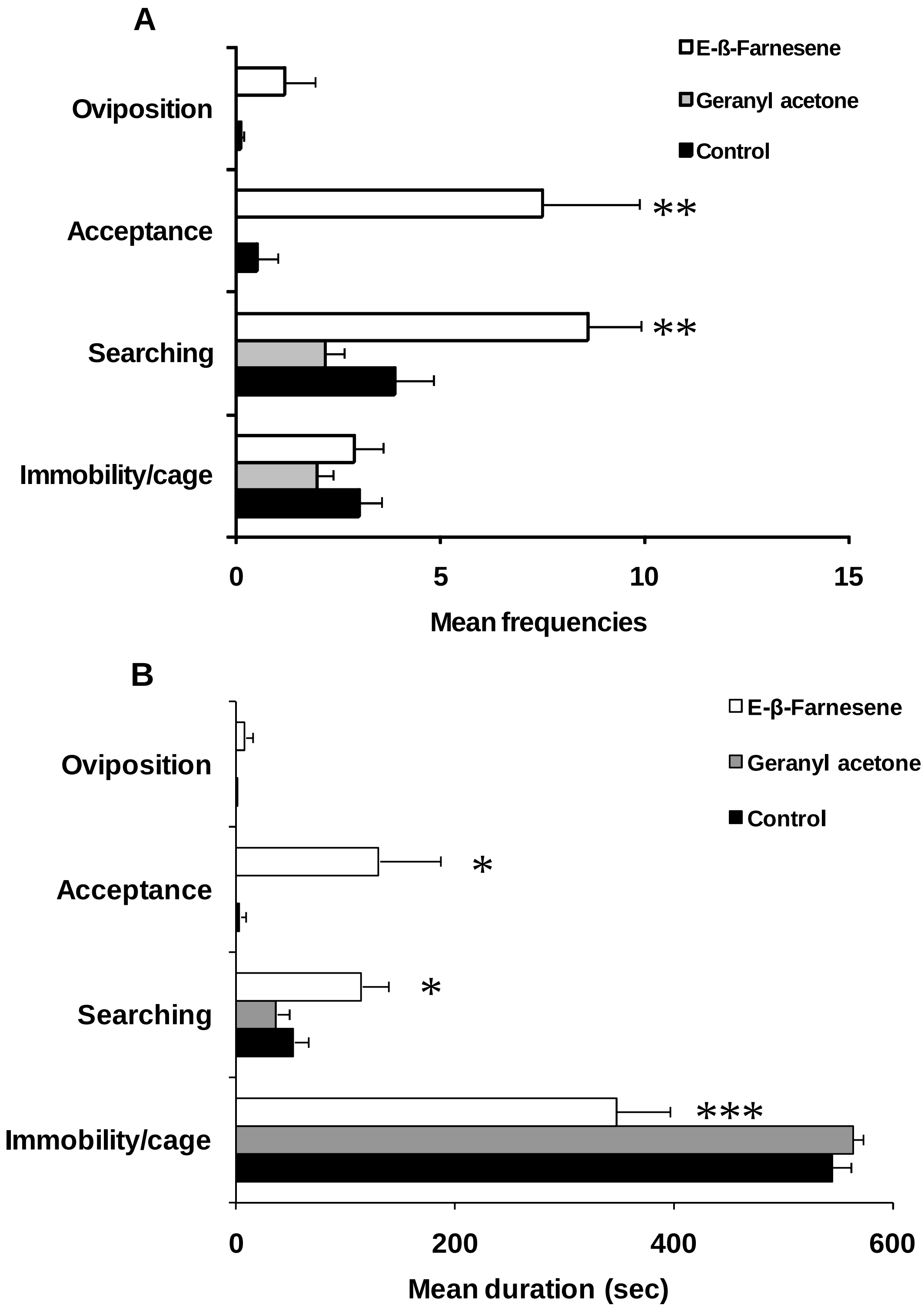

Figure 15. Mean frequencies and durations (± SE) of behavioural observations of *E. balteatus* female in response to three sources of odour: (1) (E) - β- farnesene, (2) geranyl acetone and (3) parafin solvent (as control), when presented in a rubber septum placed itself on a leaf disc system without aphids. *, **, *, indicate significant differences among the treatments when compared with control (paraffin solvent) (ANOVA, Dunnett's test at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively).**

These positive behavioural responses of *E. balteatus* females (Ovipositional tactics) to increasing aphid colony sizes, suggesting an adaptive oviposition behaviour leading the emerging larvae to locate immediately sufficient food resources and may be operating at higher colony sizes. Conversely, previous field observations showed that syrphid females preferentially oviposit in young (apterous) aphid colonies and that they avoid heavily infested plants (Kan & Sasakawa, 1986; Kan, 1988a,b; Kan, 1989). It is possible that females avoid crowded aphid colonies, since they are subjected to increased migration of the prey and therefore may not support the full development of several syrphid larvae. We think therefore that females *E. balteatus* could select quantitatively and qualitatively their oviposition site in a way that assures and optimises the developmental conditions of their offspring.

According to Bargen *et al*. (1998), the number of eggs laid by female hoverflies depends also on the presence and quantity of oviposition-eliciting substances emitted from the prey and the infested plant. Previous laboratory experiments have demonstrated that aphid colony size influence the quantity of volatile compounds emitted from aphids such as EβF (Almohamad *et al*., 2008b), and their liquid secretions such as honeydew (Budenberg & Powel, 1992; Sutherland *et al*., 2001). In our experiments, *E. balteatus* females responded positively to aphid volatiles, i.e. EβF emission from *M. persicae*. Indeed, our behavioural observations demonstrated that EβF has an attractive effect on *E. balteatus* females and acts as an oviposition stimulant. Previous experiments have showed that honeydew acts as a contact kairomone and oviposition stimulant for hoverfly females (Budenberg & Powel, 1992; Sutherland *et al*., 2001). Sutherland *et al*. (2001) also reported that syrphid females demonstrated more gustatory and oviposition responses to honeydew-treated area. These may explain our finding that *E. balteatus* females exhibited more proboscis and ovipositor extensions in response to increasing aphid colony size. With aphid alarm pheromone (EβF), honeydew might also provide hoverfly females with information about aphid colony size.

E. balteatus females did not respond positively to the presence of geranyl acetone and no egg-laying behaviour was observed in response to this molecule, neither on healthy *V. faba* leaf. This is consistent with the behavioural experiments of Francis *et al*. (2005b) using syrphid larvae. These authors showed that crushed aphids associated with *V. faba* plants were attractive to *E. balteatus* larvae but the chemical cues from healthy *V. faba* plants did not carry any infochemical role for syrphid larvae. On the other hand, although geranyl acetone did not exhibit an attractive effect towards *E. balteatus* females but this molecule may have a repellent effect with other insects. Hern & Dorn (2002) indicated that geranyl acetone acts as an inhibitor toward herbivorous insects. Indeed, volatiles substances released from healthy apple fruits (i.e. geranyl acetone) exhibited a repellent effect towards adult females of *Cydia pomonella* L. (Lepidoptera: Tortricidae).

The implications of this research are that the production of (*E*)-*β*-farnesene in response to increasing aphid colony size (Almohamad *et al.*, 2008b) and its effective kairomonal role in hoverflies attraction can potentially be used to enhance the numbers of aphidophagous syrphid in field situations. So, syrphids may be encouraged to remain in area with presence of EβF (such as from using controlled release septum containing EβF) and lay more eggs even when aphids numbers are low. This can have a significant effect even when there are low aphid densities.

In conclusion, *E. balteatus* females demonstrated a positive density-dependent response to aphid colony size in terms of foraging and oviposition behaviour. Our results also provided an opportunity to understand the mechanisms of the response to aphid colony of different sizes. Therefore, we may be better to utilise aphidophagous syrphids within an integrated pest management strategy.

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Chapter 6

Effect of intra-or interspecific competitors on the oviposition site discrimination

In the previous chapters, we demonstrated that E. balteatus females are able to select their oviposition site using different means of recognising aphid-host plant, aphid species, and aphid colony size. Other oviposition cues including the presence of intra-or interspecific competitors could be taken into account by hoverfly females during their egg-laying behaviour. The effects of such interactions may lead to a stabilisation of prey–predator populations or adversely affect foraging and oviposition performance of individual predators. These interactions probably influence the choices made by aphidophagous hoverflies. Previous studies have demonstrated that oviposition behaviour of aphidophagous predators is often modified by the presence of conspecific or heterospecific encounters sharing the same guild. Typically, this oviposition avoidance response shown by females may be considered as adaptive, a behavioural strategy enabling them to optimise their oviposition sites and maximise their fitness.

In this chapter, we decided to focus our research on the effects of intra-or interspecific interactions on the foraging and oviposition behaviour of E. balteatud females, and also to indentify the role of semiochemicals mediating these interactions. The behavioural reactions of aphidophagous hoverfly E. balteatus to the presence of conspecific larvae and their tracks in aphid patches were first investigated. Volatile chemical compounds realeased from syrphid larval tracks were identified and their behavioural impacts were also evaluated toward hoverfly females. In the second experiment, the effects of tracks left by ladybirds, (Harmonia axyridis Pallas) larvae on E. balteatus oviposition response were tested and vice-versa. Finally, the foraging and oviposition behaviour of E. balteatus was investigated in relation to the presence of aphid parasitoid (Aphidius ervi Haliday) larvae parasiting the aphid colony.

Article 7

Assessment of oviposition site quality by aphidophagous hoverflies: reaction to conspecififc larvae

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Abstract – Aphidophagous predators adapt their foraging behaviour to the presence of conspecific and heterospecific larvae. We studied the effect of the presence of conspecific larvae and their tracks on the oviposition site selection of an aphid-specific predator, *Episyrphus balteatus* DeGeer (Diptera: Syrphidae), in two-choice experiments using a leaf disc bioassay. Gas chromatography - mass spectrometry analysis was used to identify the volatile chemicals released from odour extracts of *E. balteatus* larval tracks. The behavioural effects of these volatile substances on hoverfly females were also evaluated. Our experiments demonstrated that *E. balteatus* females were deterred from ovipositing when presented with a *Vicia faba* leaf with aphids and conspecific larvae. The oviposition-deterring stimulus was also active when females were presented with a leaf that contained conspecific larval tracks. A mixture of chemical compounds was found in the volatile pattern of odour extracts of larval tracks. The main volatile chemicals were 3-methylbutanoic acid, 2-methylbutanoic acid, 2-methylpropanoic acid, 3-hydroxy-2-butanone, hexanoic acid and phenol. Females also laid significantly fewer eggs in response to odorant volatiles emitted from larval extracts. These results highlight that predatory hoverfly females avoid ovipositing in aphid colonies in which conspecific larvae or their tracks are already present, suggesting that this behaviour constitutes a strategy that enables females to optimise their oviposition site and reduce competition suffered by their offspring.

Key Words: aphid, conspecific larva, *Episyrphus balteatus*, hoverfly, larval track, *Myzus persicae*, oviposition-deterring substance, oviposition site selection, syrphid behaviour, *Vicia faba*.

Reference – Almohamad R., Verheggen FJ., Francis F., Lognay G. & Haubruge E. 2010. Assesement of oviposition site quality by aphidophagous hoverflies: reaction to conspecific larvae. *Animal Behaviour*. 79: 589-594.

Introduction

Female insects, foraging for suitable oviposition sites, often face many stimuli from their environment that ultimately influence their offspring's performance (Schoonhoven et al. 1998). Prior occupation by a conspecific individual has been found to influence females' oviposition decision in various insect species (Price 1970; van Lenteren 1980; Hemptinne et al. 1993; Janssen et al. 1995; Nufio & Papaja 2001). Typically, females avoid laying eggs on hosts that are already being exploited, which constitutes a behavioural strategy to improve the survival, growth and reproductive potential of their offspring (Kindlmann & Dixon 1993; Peckarsky et al. 2000; Almohamad et al. 2008), and to reduce competition suffered by their offspring (Doumbia et al. 1998). It has been also demonstrated that the stimuli permitting females to distinguish between the occupied and unoccupied hosts are chemical cues (Dempster 1992; Mudd et al. 1997; Seeley 1998; Li et al. 2001), which derive from conspecific eggs (Anbutsu & Togashi 1996, 1997; Scholz & Poehling 2000), larvae (Williams et al. 1986; Anbutsu & Togashi 1996; Ruzicka 1997; Doumbia et al. 1998; Ruzicka 2001; Fréchette et al. 2003) or larval tracks (Dittrick et al. 1983; Anderson et al. 1993; Yasuda et al. 2000; Michaud & Jyoti 2007).

Aphidophagous hoverflies are well-known aphid natural enemies that can have a significant impact on the suppression of aphid populations (Champers 1988). However, the stimuli that allow hoverfly females to evaluate a suitable oviposition site are still unclear. Field and laboratory observations indicate that syrphid eggs are laid close to aphid colonies (Dixon 1959; Chandler 1968a, b; Evans & Dixon 1986; Hemptinne et al. 1993). These studies showed convincingly that there is an optimal number of hoverfly eggs that should be laid in an aphid colony to maximise the number of surviving offspring, and argued that this number is typically much less than the number required to provide adequate control of aphids. Furthermore, syrphid eggs are targets for both cannibalism and intraguild predation (Branquart et al. 1997; Hindayana et al. 2001; Fréchette et al. 2007). Therefore, adult females must select oviposition sites that not only provide their offspring with sufficient resources to complete development, but also minimise their exposure to predictable sources of mortality.

Based on our previous studies (Almohamad et al. 2006, 2007, 2008; Harmel et al. 2007; Verheggen et al. 2008), we wanted to determine the cues and behavioural mechanisms that enable *E. balteatus* females to locate and select a suitable oviposition site, taking into consideration that the 'quality' of an oviposition site may depend not only on the presence of aphids and their numbers, but also on the presence of inter- or intraspecific competitors. The oviposition avoidance of occupied hosts by parasitoid females and phytophagous insects has been studied intensively (Price 1970; Rothschild & Schoonhoven 1977; van Lenteren 1981; Janssen et al. 1995; Nakashima & Senoo 2003; Kanno & Harris 2002; Li & Zhang 2006; Nakashima et al. 2006). It has been recently discovered that the oviposition behaviour of aphidophagous predators is often modified by the presence of conspecific and heterospecific larvae. Subsequently, reports appeared on the oviposition repellence of larvae or their tracks in various aphidophagous insects, initially in green lacewings (chrysopids: Růžička 1994, 1996), then in coccinellids (Hemptinne & Dixon 1991; Růžička 1997; Doumbia et al. 1998; Yasuda et al. 2000; Agarwala et al. 2003; Michaud & Jyoti 2007) and in *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae; Růžička & Havelka 1998). Nevertheless, the oviposition response of syrphid females to the presence of conspecific and heterospecific competitors has received little attention. We aimed to investigate the behavioural reaction of the aphidophagous hoverfly *E. balteatus* to the presence of conspecific larvae and their tracks in aphid patches.

Methods

Plants and insects

Broad bean plants, *Vicia faba* L., were grown in plastic trays (30 x 20 cm and 5 cm high) filled with a mix of perlite and vermiculite (1:1) and maintained in controlled-environment growth rooms (16:8 h light:dark; 20 ± 1 °C). Two aphid species, namely *Myzus persicae* Sulzer and *Megoura viciae* Buckton, were reared on *V. faba* in separated air-conditioned rooms under the same conditions as above. Adult *E. balteatus* were reared in cages (75 x 60 cm and 90 cm high) and were provided with bee-collected pollen, sugar and water. Broad beans infested with *M. viciae* were introduced into the cages for 3 h every 2 days to allow oviposition. Hoverfly larvae were mass reared in aerated plastic boxes (110 x 140 mm and 40 mm high) and were fed daily ad libitum with *M. viciae* as standard diet.

Experiment 1: Effect of conspecific larvae on oviposition

We assessed the oviposition response of *E. balteatus* females to the presence of conspecific larvae. To do so, we placed a circular piece of *V. faba* leaf, 2.5 cm in diameter, on a petri dish containing an agar solution to reduce desiccation. An excess of aphids (125 individuals) were then placed on the leaf along with two *E. balteatus* third-instar larvae. A control treatment was also run in parallel and consisted of a leaf disc infested with the same numbers of aphids without larvae. In two-choice experiments, both petri dishes containing their aphid-infested leaves (one with larvae and the other without larvae) were positioned on two Plexiglas holders, of 20 cm height, and separated by 15 cm in a net cage (30 x30 cm and 60 cm high). This set-up was previously described and shown to be an efficient method to evaluate the oviposition behaviour of *E. balteatus* in response to aphid-infested plants (Almohamad et al. 2006). A single female was then introduced into the cage and allowed to lay eggs for 3 h. The eggs laid on each leaf disc were then counted. Experiments were conducted in an air-conditioned room at 21 ± 1 °C. *Episyrphus balteatus* females were approximately 20 - 30 days old and no oviposition had been induced for 24 h prior to the experiments. Ten replicates were performed for this experiment.

Experiment 2: Effect of conspecific larval tracks on oviposition

In this experiment, two third-instar larvae of *E. balteatus* were supplied with an excess of *M. persicae* aphids (125 individuals) on a circular piece of *V. faba* leaf, 2.5 cm in diameter, placed on agar in a petri dish. Aphid-infested leaf discs were then covered with Parafilm (Pechiney Plastic Packaging, Chicago, IL, U.S.A.) and kept in the controlled incubator (16:8 h light:dark; 20 ± 1 °C) for 24 h. After 24 h, the larvae and all aphid material were carefully removed. The leaf discs (either containing larval tracks or not) were then infested with a new excess of aphids. In similar two-choice experiments as described above, a single female was introduced into the net cage (30 x 30 cm and 60 cm high) in the presence of the two aphid-infested leaf discs (one with larval tracks and the other without larval tracks as control) presented on similar 20 cm-high holders and then allowed to lay eggs for 3 h. The eggs laid on each leaf disc were then counted. Experiments were conducted in similar conditions as described above. This experiment was replicated 10 times for each treatment.

Experiment 3: Effect of conspecific larval tracks on foraging

Aphid-infested leaf discs with larval tracks were prepared as described for experiment 2. In no-choice experiments, a single *E. balteatus* female was placed in a net cage with each aphid-infested leaf disc treatment (one with larval tracks and the other without larval tracks as a control). We recorded their foraging and oviposition behaviour for 10 min using Observer version 5.0 (Noldus Information Technology, Wageningen, The Netherland). Descriptions of the four behavioural events that were observed were grouped as follows: (1) immobility: the hoverfly female was immobilised on the cage without moving; (2) searching: the hoverfly female hovered in the cage close to the infested plant; (3) acceptance: the hoverfly female landed on the plant, stayed immobile or walked on it, with proboscis extension on the plant surface; (4) oviposition: the hoverfly female showed abdomen bending and laid eggs.

The behavioural observations were conducted in an air-conditioned room at 20 ± 1 °C. Tested *E. balteatus* females were approximately 20 - 30 days old and no induction of oviposition had been realized for 24 h prior to the experiment. This experiment was replicated 10 times for each treatment.

Experiment 4: volatile chemicals released by larval tracks

Volatile collection system

We collected volatile chemicals using a push/pull volatile collection system consisting of a glass air-collection chamber (Schott, 10 cm base diameter, 12 cm high) placed in an air-conditioned room at 21 ± 1 °C, and previously washed with hot water and hexane. Ten third-instar larvae of *E. balteatus* were placed in a closed glass petri dish 5 cm in diameter without aphids, and kept inside an incubator (16:8 h light:dark; 21 ± 1 °C) for 24 h. After 24 h, the larvae were carefully removed from the glass petri dishes. Three petri dishes containing odour extracts of larval tracks were then placed in the glass air-collection chamber. Incoming air was pulled through an in-line activated charcoal filter before entering the glass chamber at a flow of 200 ml/min. The volatile-enriched air was then pulled through an adsorption trap containing 40 mg SuperQ (Alltech, Deerfield, IL, U.S.A.). Volatiles were collected during 3 h and the filters were eluted with 150 µl of diethylether. The extracted samples were directly stored in a freezer at − 80 °C before analysis. Four replicates were conducted.

Analysis of odour samples

Odour extracts of larval tracks were analysed by gas chromatography - mass spectrometry (GC - MS) for identification. The gas chromatograph (Thermo Electron Corporation, Trace GC Ultra, Interscience, Louvain-la-Neuve, Belgium) was equipped with a polar column (CP-WAX 58 (FFAP)-CB, 25 m x 0.32 mm inner diameter x 1.2 µm film thickness; Macherey – Nagel, Düren, Germany). Helium was used as the carrier gas at a pressure of 50 kPa (gas flow 1.5 ml/min). The oven temperature was programmed from 40 °C to 230 °C at a rate of 10 °C/min then to 280 °C at 30 °C/min. The mass spectra (Finnigan Traces MS, Interscience, Louvain-la-Neuve, Belgium) were operated in the EI mode (scanned mass range from 40 to 300 amu at 0.05 s/decade). Aliquots of 1 µl were injected with a splitless injector held at 280

°C. Identifications were made by comparison of retention times with those of known standards and confirmed by mass spectrometry using NIST MS Search 2.0 (National Institute of Standards and Technology, Gaithersburg, MD, U.S.A.) mass spectra database.

Experiment 5: Effect of odour extracts on oviposition

In a similar way to that outlined in experiment 4, we studied the oviposition behaviour of female hoverflies exposed to the odour extracts of larval tracks. Aphid-infested leaf discs were realised in a similar way to that described above. Disks of *V. faba* leaves were infested with 100 individuals of *M. persicae*. There were three treatments: (1) a glass petri dish containing an aphid-infested piece of leaf (treatment control), (2) a glass petri dish containing an aphid-infested piece of leaf with extracts of larval tracks, (3) a glass petri dish containing extracts of larval tracks without an aphid-infested leaf disc. In no-choice experiments, a single female was introduced into a net cage (30 x 30 cm and 60 cm high) in the presence of a Plexiglas holder supporting a glass petri dish at a height of 20 cm, and allowed to lay eggs for 3 h. The eggs laid were then counted. Ten replicates were performed for each treatment. The experiment was conducted in similar conditions as described above.

Experiment 6: Effect of amount of track extract on oviposition

Odour extracts of *E. balteatus* larval tracks were similarly prepared to that described in experiment 5 with 10 third-instar larvae of *E. balteatus* left in the petri dish for different periods of time: 30 min, 3 h, 12 h and 24 h. In similar no-choice experiments, a single female was exposed to the odour extracts and allowed to lay eggs for 3 h. The eggs laid were then counted. Ten replicates were performed for each period of time including the control (free of larval tracks).

Statistical analysis

We performed a Student’s *t* test to compare the mean number of eggs laid by *E. balteatus* females in response to the presence of conspecific larvae and their tracks. Mean behavioural observations of *E. balteatus* related to the presence of conspecific larval tracks were compared using one-way analysis of variance (ANOVA). Dunnett’s post hoc test was also used to compare the oviposition responses of *E. balteatus* females to odour extracts of conspecific larval tracks. We conducted all statistical tests using Minitab release 15.2 (Minitab Inc., State College, PA, U.S.A.).

Results

Experiment 1: Effect of conspecific larvae on oviposition

Gravid females were deterred from ovipositing in the presence of an aphid-infested leaf disc that contained conspecific larvae in terms of the total number of eggs laid (oviposition rates) compared to the control (paired *t* test: t = 4.17, P = 0.002; Figure 16).

Experiment 2: Effect of larval tracks on oviposition

Similar oviposition avoidance behaviour was also observed in response to the presence of conspecific larval tracks. Females oviposited significantly less on aphid-infested leaf discs containing conspecific larval tracks than on the control (paired *t* test: t = 4.47, P = 0.002; Figure 16).

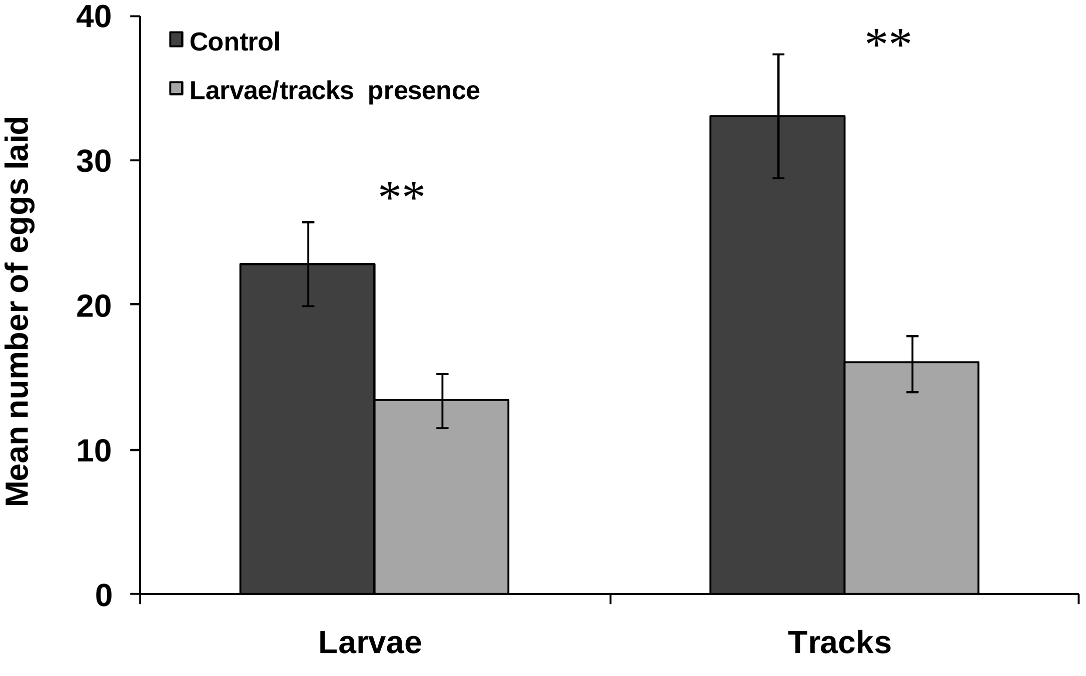

Figure 16. Mean ± SE number of eggs laid by *E. balteatus* females in response to conspecific larvae and their tracks in two choice experiments. Asterisks indicate a significant difference between treatments (Paired *t* test: P < 0.05).

Experiment 3: Effect of larval tracks on foraging

The foraging behaviour of females was also influenced by the presence of conspecific larval tracks on aphid-infested leaf discs, showing that their oviposition behaviour was less frequent (Table 6). Moreover, females spent less time ovipositing on aphid-infested leaves containing conspecific larval tracks compared to controls (one-way ANOVA: $F_{1,19} = 12.35$, $P = 0.002$). The time spent immobile also increased significantly in response to the presence of conspecific larval tracks (one-way ANOVA: $F_{1,19} = 5.24$, $P = 0.034$).

Table 6. Mean frequencies and durations ± SE of behavioural observations of *E. balteatus* female in response to conspecific larval tracks relative to those mean in control in no-choice experiments.

	Treatments		**Statistical test**		
Behavioral observations	Aphids/leaf disc with larval tracks	Aphids/leaf disc (Control)	F	*df*	*P*
	Mean observed frequencies ± SE				
Immobility/cage	2.30 ± 0.52	4.00 ± 1.23	2.97	1,19	0.102
Searching	12.20 ± 2.00	11.30 ± 2.26	0.09	1,19	0.769
Acceptance	14.60 ± 3.19	24.60 ± 2.76	4.16	1,19	0.056
Oviposition	7.00 ± 2.13	14.40 ± 1.95	6.55	1,19	0.020 *
	Mean observed duration (sec) ± SE				
Immobility/cage	225.91 ± 61.59	77.56 ± 20.23	5.24	1,19	0.034 *
Searching	138.49 ± 19.60	123.48 ± 31.25	0.17	1,19	0.689
Acceptance	195.00 ± 49.15	284.20 ± 38.14	2.06	1,19	0.169
Oviposition	40.61 ± 15.41	114.78 ± 14.42	12.35	1,19	0.002 **

Asterisks indicate a significant difference between treatments and control; one-way ANOVA: $^{*}P < 0.05$; $^{**}P < 0.01$.

Experiment 4: Volatile chemical released by larval tracks

The GC - MS analysis demonstrated the presence of volatile compounds in the larval tracks (Table 7). The major compounds released were 3-methylbutanoic acid, 2-methylbutanoic acid, 2-methylpropanoic acid, 3-hydroxy-2-butanone, hexanoic acid and phenol. Other chemical compounds, such as formic acid, butanoic acid, 2,3-butanediol, pentanoic acid, benzene ethanol, octanoic acid, ethanol, 2-(2-butoxyethoxy) and 2-ethylhexanoic acid, were also identified (Table 7).

Table 7. Analysis GC-MS of volatile chemicals (in relative %) emission from the odour of larval tracks of *E. balteatus*.

Relative amounts (area % identified chemical odor extracts of *E. balteatus* larval tracks

Volatile chemicals	***Retention time***	***Average (%)***	**min$_{obs}$ - max$_{obs}$**
Formic acid	9.83	3.67	0.00 - 12.87
3-Hydroxy-2-buthanone	11.65	12.62	0.00 - 30.35
2-methyl-propanoïc acid	16.52	13.37	7.71 - 18.92
2,3-Butandiol	16.63	1.56	0.85 - 2.02
Butanoïc acid	17.52	3.10	2.60 - 3.70
Co-elution 3-methyl butanoic acid 2-methyl butanoic acid	18.14	46.50	29.21 - 62.01
Pentanoïc acid	19.19	1.40	0.00 - 3.00
Ethanol, 2-(2-butoxyethoxy)	20.04	0.96	0.13 - 2.25
Hexanoïc acid	20.74	8.62	2.87 - 15.79
Benzene ethanol	22.13	1.08	0.00 - 3.20
2-Ethyle-hexanoic acid	22.46	0.72	0.00 - 1.49
Phenol	24.10	6.14	3.77 - 8.32
Octanoic acid	25.14	0.28	0.00 - 1.12

Experiment 5: Effect of odour extracts on oviposition

Volatile chemicals emitted from the larval tracks significantly deterred oviposition (Figure 17). Fewer eggs were laid on aphid-infested leaf discs in the presence of larval tracks (Dunnett's test: t = -4.84, P < 0.001). No egg was observed on glass Petri dishes containing larval tracks without aphids (Dunnett's test: t = -8.73, P < 0.001).

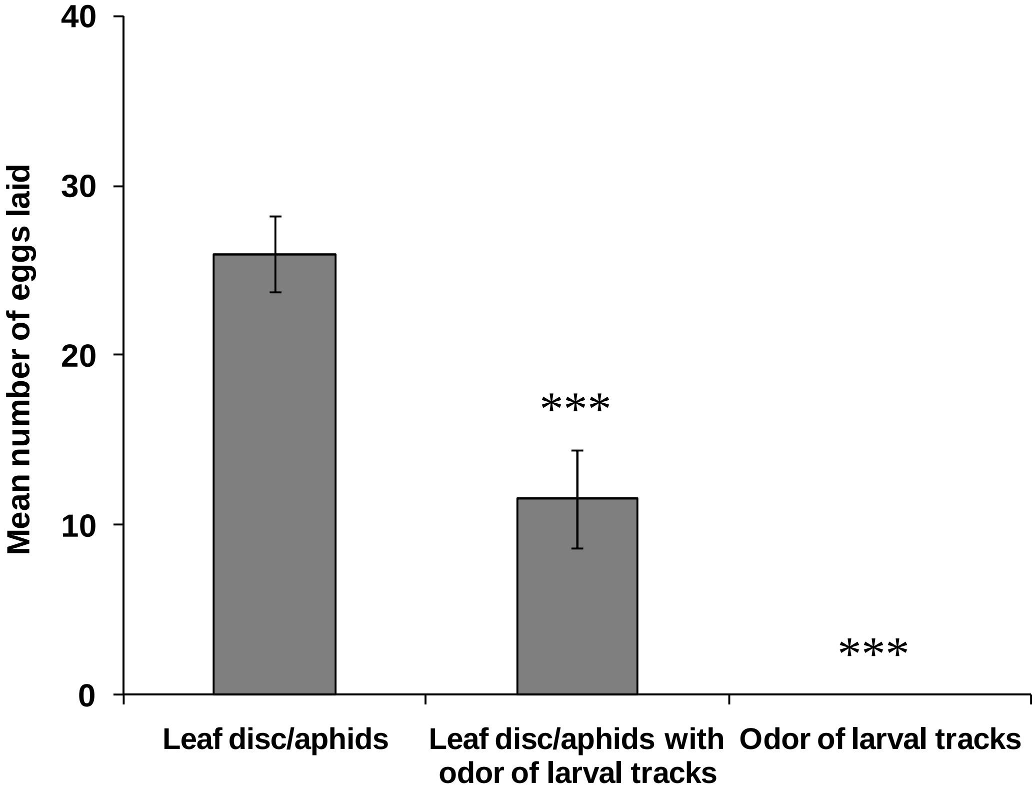

Figure 17. Mean ± SE number of eggs laid by females *E. balteatus* in response to odour of conspecific larval tracks relative to mean number laid in control in no- choice experiment. Asterisks indicate a significant difference from the control (Dunnett's test: P < 0.001).

Experiment 6: Effect of the amount of track extract on oviposition

The oviposition-deterrent effect of larval tracks was greater when large amounts of tracks were present (Figure 18). Females laid fewer eggs in the presence of tracks left after 12 h (Dunnett's test: t = -3.66, P = 0.003) and 24 h (Dunnett's test: t = -3.87, P = 0.002), but no significant difference was observed in response to larval tracks left after 30 min (Dunnett's test: t = -1.55, P = 0.354) and 3 h (Dunnett's test: t = -2.27, P = 0.094) compared to the control.

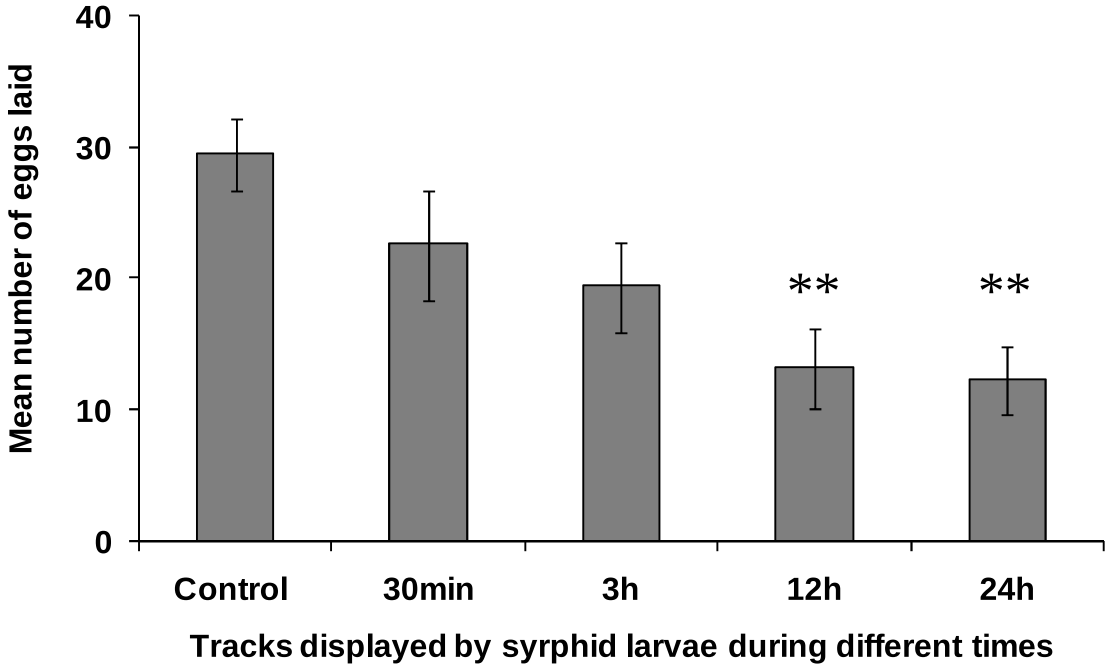

Figure 18. Mean ± SE number of eggs laid by females *E. balteatus* in response to oviposition-deterrent substances deposition by conspecific larval tracks during different periods (30min, 3h, 12h and 24h), relative to mean number laid in control in no- choice experiment. Asterisks indicate a significant difference from the control (Dunnett's test: P < 0.05).

Discussion

Episyrphus balteatus females are able to select their oviposition site by means of recognizing host plant characteristics, aphid species and the presence of heterospecific individuals (Chandler 1968b; Sadeghi & Gilbert 2000a, b; Almohamad et al. 2007, 2008a; Verheggen et al. 2008). In this study we have also shown they are able to identify the presence of conspecific competitors (i.e. larvae or their tracks).

Our experiments demonstrated that *E. balteatus* females were reluctant to oviposit on aphid-infested leaf discs that were contaminated with conspecific larvae and their tracks. Our findings are supported by previous field observations where females of the two syrphid species *Epistrophe nitidicollis* Meig and *E. balteatus* laid fewer eggs on plants that were already predated by conspecific larvae (Völkl 1990; Hemptinne et al. 1993). It is well established that females of insect predators carefully select their oviposition site to maximize their fitness (e.g. Almohamad et al. 2007, 2008a, 2009). The assessment of patch quality is critical because it determines the survival and growth rate of offspring. A female's oviposition decision would be to avoid ovipositing where competitors threaten the survival of its offspring. For example, cannibalism of eggs and first-instar larvae appears to be an important intraspecific regulation factor in aphid predator performance under rearing or application conditions, and it has been well documented in various aphidophagous species such as ladybirds (Hironori & Katsuhiro 1997; Schellhorn & Andow 1999) and hoverflies (Branquart et al. 1997; Belliure & Michaud 2001). Doumbia et al. (1998) and Yasuda et al. (2000) showed that two ladybird species, *Adalia bipunctata* and *Harmonia axyridis,* tended to oviposit less in aphid colonies in which conspecific larvae or their tracks were present, which is likely to reduce egg cannibalism. One explanation for the oviposition avoidance shown by hoverfly females in response to conspecific larvae and their tracks may be that it is induced by semiochemicals emitted by conspecific competitors. For example, previous observations have shown that parasitoids are sensitive to volatiles signalling the previous or actual presence of conspecifics or enemies (Janssen et al. 1995; Outreman et al. 2001; Nakashima & Senoo 2003; Nakashima et al. 2006). Hemptinne & Dixon (2000) extracted the larval tracks of *A. bipunctata* and found a mixture of alkanes that have multiple semiochemical functions in coccinellids including mate recognition, defence and oviposition deterrence. Indeed, Laubertie et al. (2006) later demonstrated that *A. bipunctata* females were deterred from ovipositing in response to conspecific larval semiochemicals. Strobel et al. (2008) also indicated that 3-methylbutanoic acid, 2-methylbutanoic acid and 2-methylpropanoic acid, volatile compounds released by *Oidium* sp. (an endophytic fungus), had an inhibitory effect towards many plant pathogenic fungi such as *Pythium ultimum*. In our experiment, the volatile compounds present in the tracks left by *E. balteatus* larvae did act as an inhibitor towards ovipositing syrphid females. Volatile chemical emissions from larval tracks of *E. balteatus* represent a mixture of acids, alcohols and aldehydes. Almohamad et al. (2008b) showed that these molecules were not found in the volatile pattern of odour extracts of broad bean leaf damage (aphid-free leaf disc system). This confirms that these semiochemicals were only induced by hoverfly larval tracks. As a result, the detection of these semiochemicals induced by predatory hoverfly larvae could optimize hoverfly females' searching efforts by reducing the time needed to assess aphid patch quality and consequently increase the probability of detecting a suitable oviposition site.

It has been also reported that predatory females assess, from an aphid prey patch, both the concentration of the oviposition-deterring substances and the stimulatory cues associated with aphids, and rely on the concentration of these stimuli to assess the risk of their eggs being eaten. This behaviour was clearly shown by ladybird females (Doumbia et al. 1998; Růžička 2001, 2006) and chrysopid females (Růžička 1994, 2001). In the present study, similar behavioural responses were shown by *E. balteatus* females which reduced their oviposition rates significantly in response to different amounts of oviposition-deterring substances left by conspecific larvae in aphid patches.

The oviposition avoidance of females is often accompanied by changes in their behaviour. They become more agitated and spend a greater proportion of their time walking rapidly, which leads to their departure from the patch (Price 1970; Hemptinne et al. 1992; Doumbia et al. 1998; Yasuda et al. 2000). These studies support our finding that *E. balteatus* females showed less frequent egg-laying behaviour and spent significantly less time in aphid patches contaminated with conspecific larval tracks. This pattern of behaviour could result in a wider distribution of the adults between aphid patches and may serve to reduce risks of competition among their offspring.

In conclusion, this study has highlighted that predatory hoverfly females avoid aphid colonies in which conspecific larvae or their tracks were already present. This oviposition avoidance response shown by females may be considered as adaptive, a behavioural mechanism enabling them to optimize their oviposition sites and maximize their fitness.

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Article 8

Intraguild interactions between the predatory hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) and the Asian ladybird, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae): effect of larval tracks

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Abstract – The effects of the larval tracks of *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) on the egg laying behavior of females of the predatory hoverfly *E. balteatus* were investigated in two-choice experiments. The oviposition response of *H. axyridis* to larval tracks of *E. balteatus* was also tested in one-choice experiments. Gravid *E. balteatus* females laid significantly fewer eggs on leaf discs with aphids and contaminated with tracks of conspecific or heterospecific larvae than on control leaf discs. *H. axyridis* females laid similar numbers of eggs in Petri dishes with aphids and contaminated with the tracks of *E. balteatus* larvae as in control Petri dishes. This indicates that *E. balteatus* females lay fewer eggs at sites where there are conspecific and heterospecific larval tracks, whereas the tracks of syrphid larvae did not deter *H. axyridis* females from laying eggs.

Key words: *Episyrphus balteatus*, *Harmonia axyridis*, larval tracks, *Vicia faba*, aphids oviposition-deterring substances.

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Introduction

Aphidophagous hoverflies and ladybirds are known to exploit temporary aphid colonies as food resources and significantly suppress aphid abundance (Dixon, 1985; Chambers & Adams, 1986; Dixon et al., 1997; Lee & Kang, 2004). It has been also demonstrated that the survival of the larvae of these aphid predators and their efficiency in reducing aphid populations mainly depends on the quality and the quantity of the patches of aphid prey (Kan, 1988; Kindlmann & Dixon, 1993; Hemptinne et al., 1993; Almohamad et al., 2007, 2008). The selection of oviposition sites by gravid females should therefore reflect a preference for patches of prey of high nutritional value and/or where the risks of predation and competition are low, especially in insect species where neonate offspring have limited mobility and are therefore relatively sessile (e.g. syrphid larvae, Chandler, 1969).

The suitability of an oviposition site depends not only on the number of aphids present at the time of oviposition, but also on the presence of intra- or inter-specific competitors. Several authors have shown that ovipositing insect predators respond to chemical cues (i.e. oviposition-deterrents) indicating that a prey patch is already being exploited by conspecific larvae (Růžička, 1994, 1996, 1997; Doumbia et al., 1998; Růžička & Havelka, 1998; Yasuda et al., 2000; Oliver et al., 2006; Michaud & Jyoti, 2007). Most of these studies have focused on coccinellids and chrysopids, with studies on syrphids very recent and scarce (e.g. Scholz & Poehling, 2000; Pineda et al., 2007; Almohamad et al., 2008; Putra et al., 2009).

Larvae of predatory hoverflies and ladybirds occur on the same aphid infested plants and strongly interact with each other when their aphid prey becomes scarce (e.g., Agarwala & Yasuda, 2001). The Multicoloured Asian Ladybird, *H. axyridis* is native to South-East Asia (e.g. Dobzhansky 1933; Kuznetsov 1997). It was introduced as biological control agent in Belgium in 1997 and by 2006 was recorded in all regions of Belgium (Adriaens et al., 2003). The hoverfly *E. balteatus* is usually the most abundant syrphid aphid predator in Europe, where it naturally occurs in high numbers in numerous crops (Tenhumberg & Poehling, 1991; Gilbert, 1993; Colignon et al., 2001; Miñarro, 2005). These two aphid predators are known to be important biocontrol agents of aphid populations (Chambers & Adams, 1986; Chambers, 1988; Koch, 2003; Roy et al., 2006; Pineda & Marcos-García, 2008).

Pell et al. (2008) have reported that *H. axyridis* remains in equilibrium with its co-evolved native guild of predators through niche complementarity, but in its exotic range the native guild of predators have co-evolved with each other but not with *H. axyridis.* Recent observations also suggest that this species is now invading (semi-)natural ecosystems in Belgium and is a potential threat to native ladybird species and other aphid predators (Adriaens et al., 2003). Field observations have shown that *H. axyridis* and *E. balteatus* often coexist in aphid colonies in different habitats in Belgium-Gembloux (Alhmedi et al., 2007). The objective of this study was to obtain a better understanding of the mechanisms that shape guilds of aphidophagous predators and their potential use in biocontrol. In particular, the effect of the larval tracks of *E. balteatus* and *H. axyridis* on oviposition-site selection by *E. balteatus* females was detrmined. The oviposition response of *H. axyridis* females to tracks left by *E. balteatus* larvae was also investigated.

Materials and methods

Plants and insects rearing **-** Broad-bean plants (*Vicia faba* L.) were grown in 30cm x 20cm x 5cm plastic trays filled with a mixture of perlite and vermiculite (1/1) and maintained in controlled-environment growth rooms (16:8 Light: Dark ; 20 ± 1°C). Two aphid species (*Myzus persicae* Sulzer and *Megoura viciae* Buckton) were reared on broad-bean plants in separate rooms. Adult *E. balteatus* were reared in 75 x 60 x 90 cm cages provided with bee-collected pollen, sugar and water. Broad-bean plants infested with *M. viciae* were introduced into the cages for 3 h every two days for the adults to lay their eggs on. Hoverfly larvae were mass-reared in aerated plastic boxes (110 x 140 x 40 mm) and fed daily *ad libitum* with *M. viciae*.

H. axyridis adults were obtained from Montreal Canada (Eric Lucas laboratory, University du Québec à Montréal). Adults and larvae were then mass-reared in aerated plastic boxes (up to 25 individuals per container) and fed daily *ad libitum* with *M. viciae*. Bee-collected pollen, sugar and water were also provided. Boxes were placed in controlled-environment incubators (25 ± 2°C; 70 % RH and photoperiod 16L: 8D). Mated and fertile females used in the experiment were isolated individually in separate boxes; no oviposition occurred in the 24h prior to experimentation.

EXP 1. Effect of conspecific and heterospecific larval tracks on syrphid oviposition

This experiment was conducted using aphids on leaf discs (Almohamad et al., 2006). A 2.5cm-diameter circular piece of *V. faba* leaf was cut and placed in a Petri dish containing agar solution (7 ml agar, 1% solution w:w), which increased the humidity and prevented the leaf disc from drying out. Prior to use, either two third-instar larvae of *E. balteatus,* or two fourth-instar larvae of *H. axyridis* were confined with an excess of *M. persicae* (125 individuals) on each leaf-disc by parafilm paper (Parafilm®, Pechiney Plastic Packaging, Chicago, USA) and kept in the controlled incubator (16:8 Light: Dark; 20 ± 1°C) for 24 h. After 24h the larvae and all aphid material were carefully removed. Then, each leaf disc with the tracks left by syrphid or coccinellid larvae was infested with an excess of recently collected *M. persicae* (125 individuals). The control consisted of a leaf disc infested with the same numbers of aphids but lacking larval tracks. In a two-choice experiment, both treated and control leaf-discs (i.e. with and without larval tracks) were positioned on two Plexiglass holders 20-cm high, separated by 15 cm, in a net cage (30 x 30 x 60 cm). This arrangement was previously used to evaluate the oviposition response of *E. balteatus* to aphid-infested plants (Almohamad et al., 2006). A single female was then introduced into the cage and allowed to lay eggs for 3 hours. The number of eggs laid on each leaf disc was then counted. These experiments were conducted in an air-conditioned room at 21 ± 1°C. *Episyrphus balteatus* females were approximately 20-30 days old, and had not oviposited during the previous 24 hrs. There were ten replicates of each treatment (one assessing the control leaf discs vs. those with larval tracks of *E. balteatus*, and the other control leaf discs vs. those with larval tracks of *H. axyridis*).

EXP 2. Effect of tracks of syrphid larvae on H. axyridis oviposition

Ten third-instar hoverfly larvae were supplied with an excess of a mixture of different instars of *M. viciae* aphids (150 individuals) in a 9cm-diameter glass Petri dish, the base of which was covered with filter paper. After 24h the larvae and all aphid materials were carefully removed. In a no-choice experiment, a single mated *H. axyridis* female was then placed in each dish with an excess of aphids (150 individuals) and the number of eggs laid during the next 24 hrs was recorded. The control consisted of 10 females kept individually in clean glass Petri dishes that contained a similar number of aphids. The experiment was repeated 10 times for each treatment.

Statistical analysis

A paired t-test was used to compare the mean number of eggs laid by the aphid predators in the two-choice experiments. In the one-choice experiment, the means were compared using a two-sample t-test. All statistical tests were conducted using Minitab® release 15.2.

Results

EXP 1. Effect of conspecific and heterospecific larval tracks on syrphid oviposition

Gravid *E. balteatus* females laid significantly fewer eggs on leaf discs on which there were tracks of either conspecific hoverfly larvae (paired t-test, t = 4.47, P = 0.002) or heterospecific ladybird larvae (paired t-test, t = 3.06, P = 0.014), by their respective values (16.00 ± 1.99; 20.50 ± 3.10) compared to the controls by their respective values (33.10 ± 4.29; 36.00 ± 4.60) (Figure 19). Thus the presence of conspecific and heterospecific larval tracks deterred gravid female *E. balteatus* from ovipositing.

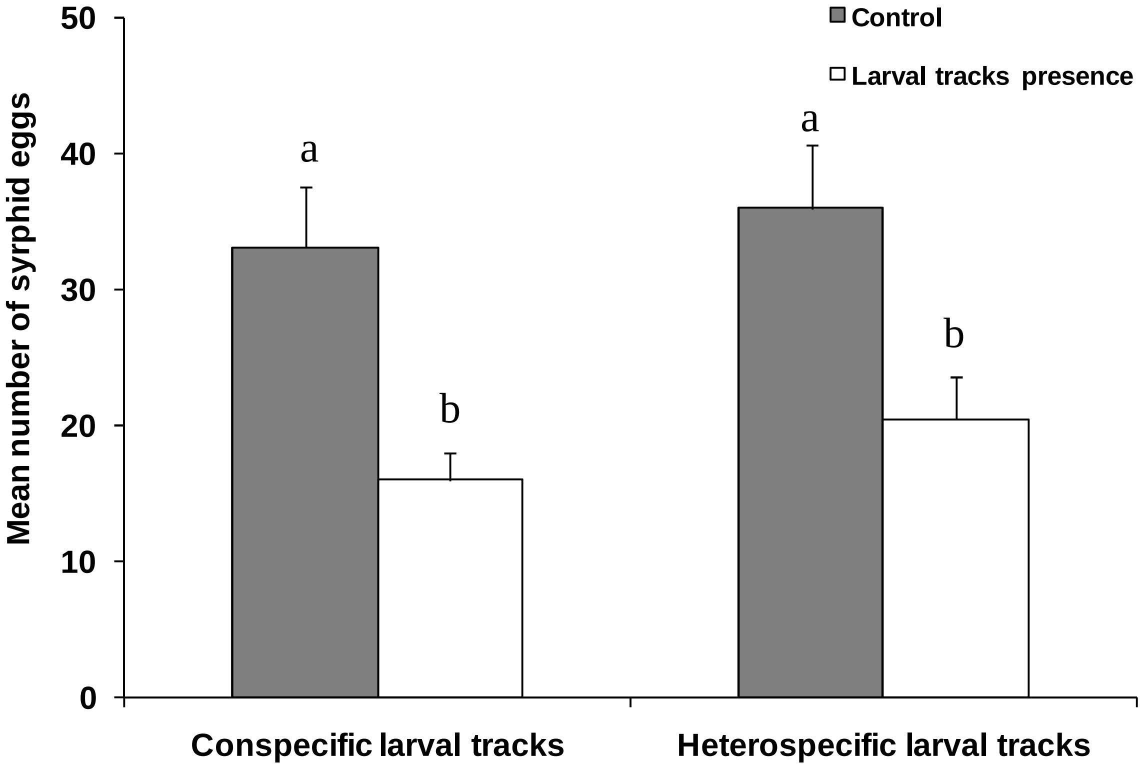

Figure 19. Mean (± SE) number of eggs laid by *E. balteatus* females on leaf discs contaminated with the tracks of larvae of their own species or that of larvae of ladybird *H. axyridis* compared a control in two-choice experiments. Means with different letters are significantly different (Paired t-test, P < 0.01).

EXP 2. Effect of tracks of syrphid larvae on H. axyridis oviposition

Compared to the control (15.50 ± 3.10), gravid *H. axyridis* females were not deterred from ovipositing by the tracks of *E. balteatus* larvae (15.80 ± 3.20) (two-sample t-test, t = -0.07, P = 0.947, *df* = 18) (Figure 20).

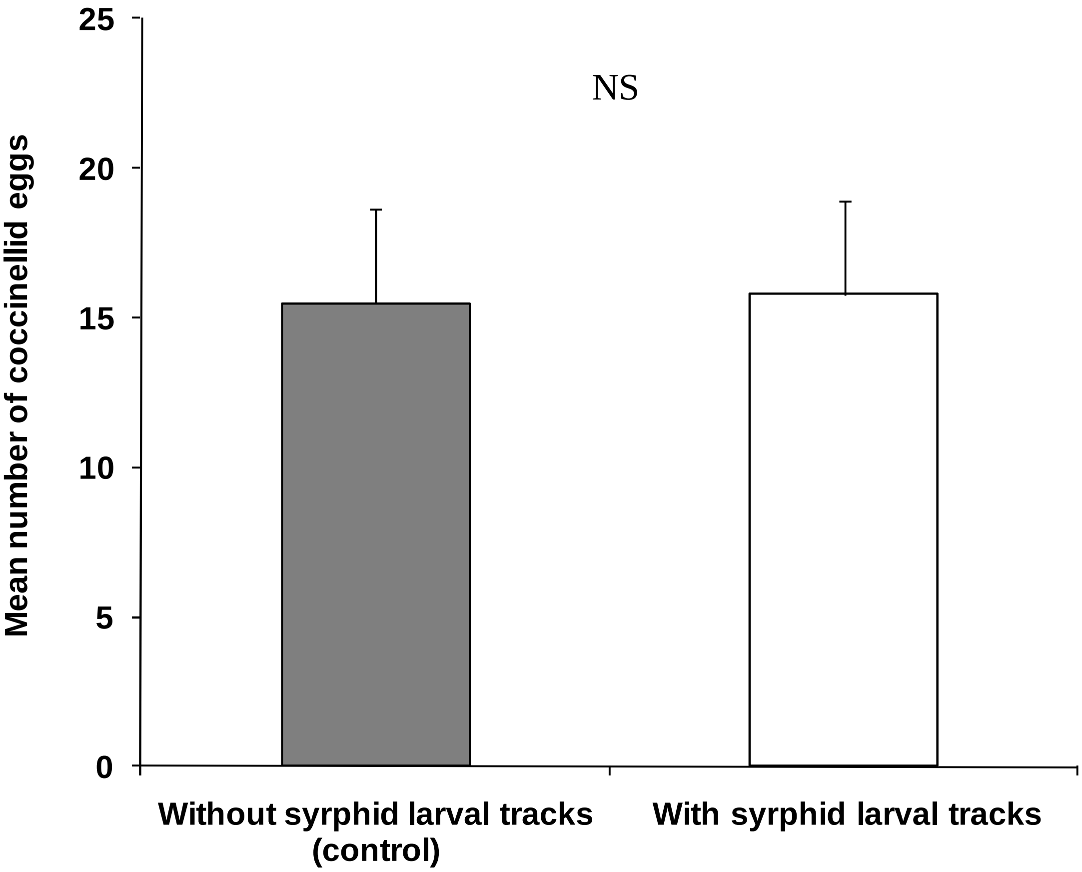

Figure 20. Mean (± SE) number of eggs laid by *H. axyridis* in Petri dishes containing aphids and contaminated with the tracks left by *E. balteatus* larvae compared with the average number laid in the control (clean Petri dishes containing only aphids). NS indicates no significant difference between treatments (two-sample t-test, P > 0.05).

Discussion

Adults of predators, the larvae of which compete for the same trophic resource, can reduce the intensity of inter-specific competition between their larvae by avoiding food resources contaminated with heterospecific semiochemicals (e.g. Růžička, 2001a,b). Several studies have reported lower frequencies of inter- vs. intra-specific interactions in phytophagous insects (Birch et al., 1980; Byers et al., 1984; Thiéry et al., 1992) and parasitoids (Janssen et al., 1995). In insect predators, interspecific interactions occur between chrysopids (Růžička, 1996, 1998) and coccinellids (Růžička, 2001a,b). This study records for the first time interspecific interactions between hoverflies and coccinellids.

In our experiments, *E. balteatus* females laid fewer eggs when oviposting in aphid colonies contaminated with the tracks of both conspecific larvae and heterospecific coccinellid larvae. A similar response is also shown by female *Coccinella septempunctata* L. to tracks left by larvae of both *C. septempunctata* and *Chrysopa oculata* Say (Růžička, 1997). Putra et al. (2009) also report that hoverflies tend to reduce their rate of laying eggs when ladybird larvae are present. The mechanism underlying the reduction in oviposition by *E. balteatus* females in the presence of the tracks or larvae of both conspecific and heterospecific predators is unknown. This response is likely to be adaptive as laboratory studies have demonstrated that *H. axyridis* larvae will eat *E. balteatus* eggs (Alhmedi et al., unpublished data) and syrphid larvae (Putra et al., 2009).

H. axyridis females are deterred from ovipositing on broad-bean plants contaminated with conspecific larval tracks but not those of *C. septempunctata* larvae (Yasuda et al., 2000). The no-choice tests used here indicate that *H. axyridis* females exhibit a weak response to the presence of oviposition-deterring substances in the tracks of *E. balteatus* larvae (10 replicates only). It is interesting to speculate why adult ladybirds respond only to conspecific cues. It is likely that each species of ladybird is associated mainly with one particular habitat (Honěk, 1985), where it is more likely to meet individuals of its own species than of other species.

It is well established that females of insects occupying the third trophic level (i.e. parasitoids and predators) avoid ovipositing where competitors threaten the survival of their offspring by responding to the semiochemicals released by conspecific competitors. For example parasitoids are sensitive to volatiles signaling the previous or actual presence of conspecifics and to chemical trails deposited by their enemies (Price, 1970; Janssen et al., 1995; Nakashima et al., 2004). Several aphidophagous and coccidophagous predators respond to oviposition-deterring semiochemicals in the tracks left by conspecific larvae (Merlin et al., 1996; Doumbia et al., 1998; Hemptinne et al., 2001; Růžička, 2002, 2006). In our experiments, *E. balteatus* females laid fewer eggs on leaf discs contaminated with the tracks of conspecific and heterospecific larvae, possibly by responding to a chemical(s) in the tracks (i.e. oviposition – deterrents). Such responses enable females to quickly assess the quality of patches of prey and enhance their probability of discovering suitable oviposition sites.

E. balteatus and *H. axyridis* have been used to control aphids in greenhouses (Snyder et al., 2004; Pineda & Marcos-García, 2008). The present study provides new information on semiochemical spacing of these aphidophagous predators over prey patches of different quality. This knowledge could be employed to improve the effectiveness of these aphid predators when used in biological control programs.

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Article 9

Discrimination of parasitised aphids by a hoverfly predator: effects on larval performance, foraging, and oviposition behaviour

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Abstract – The choice of oviposition site by female aphidophagous predators is crucial for offspring performance, especially in hoverflies whose newly hatched larvae are unable to move over large distance. Predator and parasitoid interactions within the aphidophagous guild are likely to be very important in influencing the choices made by predatory hoverfly females. In the present study, the foraging and oviposition behaviour of the aphidophagous hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) was investigated with respect to the parasitised state of its aphid prey, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), that were parasitised by*Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). We also recorded the number of eggs laid by hoverfly females when subjected to parasitised aphids. Furthermore, we studied the influence of being fed with parasitised aphids on hoverfly larval performance. Hoverfly females did not exhibit any preference for plants infested with unparasitised or aphids parasitised for 7 days. On the other hand, plants infested with mummies or exuvia were less attractive for *E. balteatus*. These results were correlated with (i) the number of eggs laid by *E. balteatus* females and (ii) larval performance. Thus, our results demonstrate that *E. balteatus* behaviour is affected by parasitoid presence through their exploitation of aphid colonies. Indeed, hoverfly predators select their prey according to the developmental state of the parasitoid larvae.

Key words: intraguild competition, *Episyrphus balteatus*, Diptera, Syrphidae, parasitoid, *Aphidius ervi*, Hymenoptera, Aphidiidae, pea aphid, *Acyrthosiphon pisum*, Homoptera, Aphididae

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Introduction

Aphid communities are subjected to predation by a broad range of specialist and generalist arthropod predators and parasitoids, whose number and variety fluctuate according to host plant species and phenology, season, and weather conditions. Natural enemies of aphids, such as hoverflies (Gilbert, 1986), coccinellids (Hodek & Honek, 1996; Verheggen et al., 2007), lacewings (Principi & Canard, 1984), midges (Nijveldt, 1988), spiders (Sunderland et al., 1986), and parasitoids (Stáry, 1970) are major components of the predatory guild associated with aphid colonies.

Among these natural enemies, intraguild predation tends to be asymmetrical, with larger individuals acting as ‘superpredators’ and smaller individuals being the intraguild prey (Lucas et al., 1998). The effects of such interactions may lead to a stabilisation of prey–predator populations (Hanski, 1981; Godfray & Pacala, 1992) or adversely affect foraging and oviposition performance of individual predators (Polis et al., 1989; Hemptinne et al., 1992; Rosenheim et al., 1995; Ruzicka, 1996). These intraguild interactions probably influence the choices made by aphidophagous female hoverflies (Gilbert, 2005).

The influence of parasitism on prey discrimination by the predatory hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) was studied in the present work. The larvae of this species are predators of more than 100 species of aphids worldwide (Sadeghi & Gilbert, 2000b). Although many aphidophagous hoverflies are generalists, previous studies indicated that they are selective in their prey choice (Sadeghi & Gilbert, 2000a,b; Almohamad et al., 2007) and that they can forage in an optimal way (Hemptinne et al., 1993; Almohamad et al., 2007; Harmel et al., 2007). For polyphagous syrphids, such as *E. balteatus*, the choice of oviposition site has an important effect on offspring performance, as syrphid larvae have rather limited dispersal abilities (Chandler, 1969).

In the current study, *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) was used as parasitoid and the pea aphid *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) as the prey/host. This parasitoid has great potential for successful aphid control because of its short development time, high fecundity, and high dispersal capacity (Rabasse & van Steenis, 1999).

Previous laboratory and field studies have suggested that spatial population dynamics, foraging behavior, and oviposition decisions of aphid parasitoids and predators are determined by the density, distribution, and quality of aphid colonies (Cappuccino, 1988; Morris, 1992; Mackauer & Völkl, 1993; Müller & Godfray, 1999a,b). Thus, most natural enemies compete for the same prey/host (Polis et al., 1989) and tend to aggregate in aphid patches (Frazer, 1988), thereby creating favorable situations for intra and interspecific encounters. In several documented cases exploring predator–parasitoid interactions, generalist predators attacked parasitised hosts, consuming both the host and the immature parasitoid developing inside the host (Ruberson & Kring, 1991; Hoelmer et al., 1994; Meyhöfer & Hindayana, 2000). Additionally, discrimination between parasitised and unparasitised prey could also enable foraging predators to evaluate prey and patch quality. According to Rosenheim et al. (1995), few studies have described this discrimination behaviour in predators and none has discussed its functional significance. In the present study, we investigated the foraging and oviposition behaviour of *E. balteatus* in relation to the presence of *A. ervi* larvae parasitising the aphid colony. The effects of parasitised aphids as food on the fitness of *E. balteatus* larvae were also investigated.

Materials and methods

Plant and insect rearing – Broad beans [*Vicia faba* L. (Fabaceae)] were grown in 30×20×5 cm plastic trays filled with a mix of perlite and vermiculite (1:1) and maintained in controlled environmental growth rooms (L16:D8 and 20 ± 1°C). Two aphid species, namely, *Megoura viciae* Buckton and *Acyrthosiphon pisum* Harris (both Homoptera: Aphididae), were taken from stock rearing on *V. faba*, in separate air-conditioned rooms under the same conditions as above. Adult *E. balteatus* were reared in 75 × 60 × 90 cm net cages and were provided with bee-collected pollen, sugar, and water. Broad beans infested with *M. viciae* were introduced into the cages for 3 h every 2 days to allow oviposition. Hoverfly larvae were mass reared in aerated plastic boxes (110 × 140 × 40 mm) and were fed daily ad libitum with *M. viciae* as standard diet. *A. pisum* was used as *E. balteatus* prey or *A. ervi* host. In order to obtain parasitised aphids and mummies, 150 aphids were introduced into a Petri dish (9 cm in diameter). Three previously mated parasitoid females were released into the Petri dish and kept with the aphid colony for 4 h. This method allowed us to obtain 91 ± 2% parasitised aphids (mean ± SE). Parasitised aphids were subsequently placed on broad beans for 7 days, and will be referred to as parasitised aphids. Mummies were obtained 10–12 days after the parasitoid infestation.

Oviposition preference

***Influence of parasitised aphids on hoverfly behaviour* –** In no-choice experiments, a single *E. balteatus* female was placed in a net cage (30 x 30 x 60 cm) with a 20 cm-tall *V. faba* plant infested with parasitised *A. pisum* having different development stages of parasitoid larvae.

Three developmental stages of parasitoid larvae and one control (unparasitised *A. pisum*) were tested: (i) parasitised *A. pisum* after 7 days, (ii) mummified *A. pisum*, and (iii) exuvia of mummies. The female foraging behavior was recorded for 10 min using the Observer® (version 5.0; Noldus Information Technology, Wageningen, The Netherlands). Descriptions of the four behavioural events that were observed are presented in Table 1. Behavioural observations were conducted in an air-conditioned room at 20 ± 1°C. *E. balteatus* females tested were approximately 20–30 days old and no induction of oviposition had occurred for 24 h prior to the experiment. This experiment was replicated 10 times for each treatment.

Influence of parasitised aphids on hoverfly oviposition rate – In similar no-choice experiments, a single *E. balteatus* female was placed in a net cage and was allowed to lay eggs in the presence of an aphid colony. Three developmental stages of parasitoid larvae and one control (unparasitised *A. pisum*) were tested: (i) parasitised *A. pisum* after 7 days, (ii) mummified *A. pisum*, and (iii) exuvia of mummies. The number of eggs (oviposition rate) was counted after 3 h. Experiments were conducted in an air-conditioned room at 20 ± 1 °C. *Episyrphus balteatus* females were approximately 20–30 days old and no induction of oviposition had occurred for 24 h prior to the experiment. These experiments were repeated 10 times for each stage of parasitism.

Larval performance

To assess the effect of parasitized aphids as food on *E. balteatus* larval performance, 20 second instars were weighed and placed individually in plastic Petri dishes (9 cm in diameter). Each day, the larvae were fed ad libitum with either unparasitised *A. pisum* or parasitised *A. pisum* (aphids parasitised for 7 days according to the same method as presented above). Among these 20 larvae, six larvae per treatment (unparasitised and parasitised *A. pisum*) were observed daily to estimate their food consumption, defined as the difference between the weight of the food supplied and the weight of the food consumed. The weight gained by these second instars was also measured as the difference between the weight of second instars at the beginning of the experiment and weight on the day following pupation. Additionally, mummified *A. pisum* were used as food and the impact on larval performance was tested. Ten second and seven third instars fed ad libitum with mummified *A. pisum* were observed daily in plastic Petri dishes (9 cm in diameter). Observations were made daily until the larvae died or developed into adults. Hoverfly larvae were kept in an incubator at 20 ± 1°C and L16:D8 photoperiod. The duration of their development, survival rates, food consumption, and weight gain were determined. The pupae and the adults were also weighted (using a Sartorius microbalance Mc5; Labo and Co, Mandres-les-Roses, France).

Statistical analysis

Mean frequencies of behavioral events and durations were compared using analysis of variance (ANOVA; general linear model) and Dunnett's test, conducted with Minitab® software (version 12.2; Minitab Inc, State College, PA, USA). In cases of heterogeneity of variables demonstrated by Bartlett's test, data were log transformed before parametric tests. Percentages of survival rate of hoverfly larvae were compared using $\chi 2$-test.

Results

Oviposition preference

Influence of parasitised aphids on hoverfly behaviour **–** The mean frequencies and mean durations observed for each *E. balteatus* behavioural event according to the developmental stage of the parasitoid larvae are presented in Figure 21. A and B, respectively. Hoverfly oviposition behaviour was significantly affected by the parasitized state of its aphid prey, both in terms of frequencies (ANOVA: $F_{3,39} = 16.61$, $P < 0.001$) and durations (ANOVA: $F_{3,39} = 20.27$, $P < 0.001$). Similar results were obtained when observing acceptance behaviour; the mean frequency (ANOVA: $F_{3,39} = 12.61$, $P < 0.001$) and duration (ANOVA: $F_{3,39} = 6.28$, $P = 0.002$) of this behavioural event were significantly affected by the presence and development stage of a parasitoid larva inside the prey. The presence of a 7-day-old parasitoid larva inside the aphid prey did not affect the foraging behaviour of the predator *E. balteatus*. Indeed, the hoverfly predator showed similar acceptance of a plant infested by unparasitised aphids or aphids containing a 7-day-old parasitoid larva, either in terms of frequencies (Dunnett's test: $T = 2.336$, $P = 0.069$) or durations (Dunnett's test: $T = 0.247$, $P = 0.989$) of the corresponding observed behaviour. In the presence of a plant infested with unparasitised prey, the *E. balteatus* female showed a short period of immobility, and this behavior remained unchanged with parasitized aphids (Dunnett's test: $T = 0.225$, $P = 0.992$). However, when presenting a *V. faba* infested with mummies, the hoverfly stayed immobile for a longer period (Dunnett's test: $T = 4.039$, $P = 0.001$). Similar observations were made with exuvia of mummies as 'prey' (Dunnett's test: $T = 4.145$, $P<0.001$).

Influence of parasitised aphids on hoverfly oviposition rate **–** The number of eggs laid by *E. balteatus* females was significantly affected by the presence of parasitoid pupae inside the aphid prey (Figure 22). Indeed, whereas hoverfly predators did not distinguish between unparasitized and parasitized aphids in terms of number of laid eggs (Dunnett's test: T = 1.335, P = 0.414), the oviposition rate was reduced when presenting mummified aphids (Dunnett's test: T = – 4.684, P < 0.001), and even more reduced when presenting exuvia (Dunnett's test: T = –8.096, P < 0.001) to the hoverfly female.

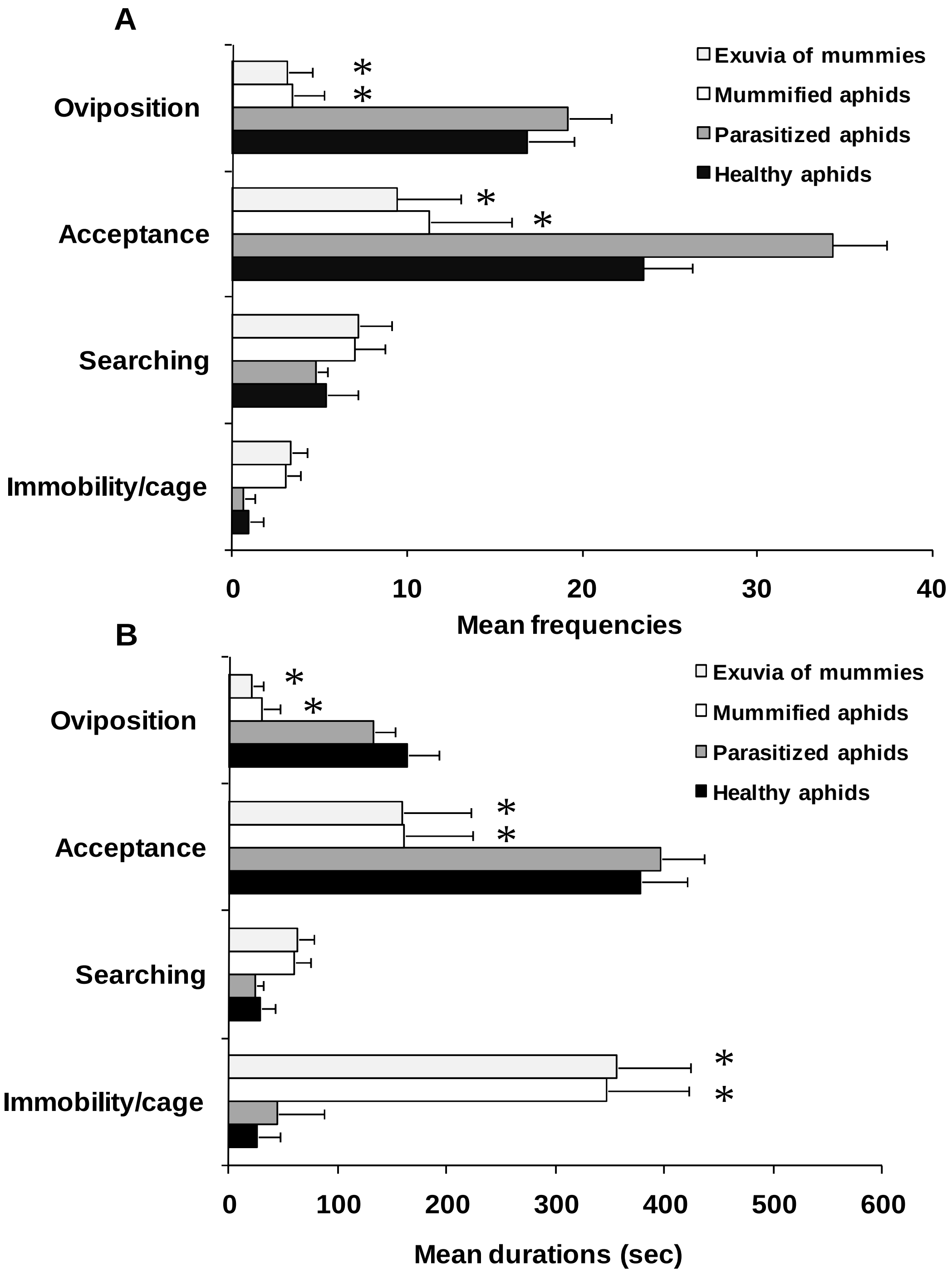

Figure 21. Effects of parasitised *A. pisum* on the oviposition behaviour of female *E. balteatus* in relation to developmental stage of the parasitoid. (A) Mean frequencies (± SE) of behavioural events of hoverfly females. (B) Mean durations (± SE) of behavioural events of hoverfly females. * indicates a significant difference among the treatments when compared with control (unparasitised aphids) [analysis of variance (ANOVA), Dunnett's test: P < 0.05].

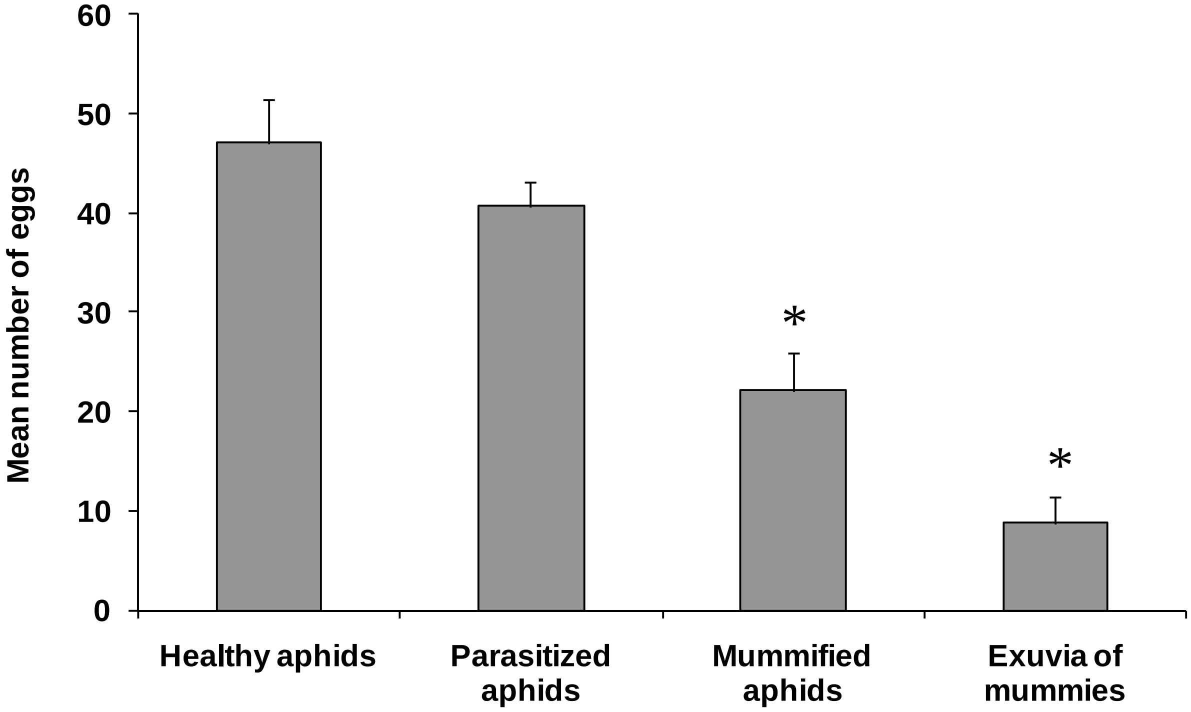

Figure 22. Effects of parasitised aphid *A. pisum* on oviposition rate of *E. balteatus* females in relation to developmental stage of the parasitoid. * indicates a significant difference among mean number of eggs laid (± SE) when compared to the control (unparasitised aphids) [analysis of variance (ANOVA), Dunnett's test: $P < 0.05$].

Larval performance

Several parameters concerning the development, growth, and survival of second instars, pupae, and adults of *E. balteatus* have been compared for hoverflies fed with aphids at three levels of parasitism (i.e., unparasitised aphids, aphids infested with a 7-day-old parasitoid larva, and mummies). No data on mummified aphids were obtained, as they were not consumed by the hoverfly larvae. *Episyrphus balteatus* larvae developed successfully to maturity with unparasitized or parasitised *A. pisum*. No difference in survival of *E. balteatus* second instars fed with unparasitised or parasitised pea aphid was observed ($\chi 2 = 0.06$, d.f. = 1, and $P = 0.801$). Most of these larvae pupated and most of the resulting pupae developed into adults. There was no significant difference in development time between larvae fed with unparasitised and parasitised *A. pisum* (ANOVA: $F_{1,33} = 0.03$, $P = 0.873$). Additionally, no difference in pupal weight was observed (ANOVA: $F_{1,33} = 2.37$, $P = 0.134$) (Table 8). However, the adults resulting from larvae fed with unparasitised *A. pisum* were significantly heavier (ANOVA: $F_{1,28} = 9.57$, $P = 0.005$). Although weight-gain in second instars did not differ significantly when fed with unparasitised and parasitised aphids (ANOVA: $F_{1,33}$ =

2.92, P = 0.097), hoverfly larvae consumed a smaller amount of parasitised aphids than unparasitised aphids (ANOVA: $F_{1,11} = 9.35$, P = 0.012) (Table 8).

We also found that second-instar hoverflies fed with mummified aphids did not develop into pupae, because they did not consume the mummified aphids. Similarly, we found that hoverfly third instars pupated rapidly and did not exploit the mummified aphids as food either. The weight of hoverfly third instars did not differ significantly on the day following pupation when compared to the weight of third instars at the beginning of the experiment (ANOVA: $F_{1,13} = 2.00$, P = 0.183).

Table 8. Effects of parasitised aphids *A. pisum* as food on various performance parameters of *E. balteatus* (mean ± SE).

Parameter	***A. pisum*** Unparasitised	Parasitised	Statistical test	
Second instar larva to pupa developmental time (days)	4.44 ± 0.22	4.50 ± 0.27	$F_{1,33} = 0.03$	P = 0.873
Percentage of survival (from second instar larva to adult emergence)	75.00 ± 7.07	70.00 ± 14.14	$X^2_1 = 0.02$	P = 0.888
Pupal weight (mg)	39.07 ± 1.32	36.29 ± 1.21	$F_{1,33} = 2.37$	P = 0.134
Pupal development to adult (days)	8.13 ± 0.09	8.21 ± 0.21	$F_{1,28} = 0.13$	P = 0.724
Adult weight (mg)	27.03 ± 0.85	23.36 ± 0.83	$F_{1,28} = 9.57$	P = 0.005
Weight gain of second instar larvae (mg)	32.50 ± 1.27	29.44 ± 1.22	$F_{1,33} = 2.92$	P = 0.097
Food consumption of second instar larvae per day (mg)	105.42 ± 5.60	87.71 ± 1.46	$F_{1,11} = 9.35$	P = 0.012

Discussion

In a natural environment, most aphidophagous hoverflies feed on a wide range of prey species (Rojo et al., 2003) that are not necessarily of similar nutritional value (Sadeghi & Gilbert, 2000b; Almohamad et al., 2007). As predicted by optimal foraging models, predators searching for prey should select the most profitable prey and reject unprofitable ones (Crawley & Krebs, 1992). Such decisions minimise time loss and maximise energy return (Stephens & Krebs, 1986). Therefore, if parasitism alters prey suitability and profitability, the detection and recognition of chemical marks left by a parasitoid female, or of morphological and physiological changes provoked by the developing immature parasitoid, would have strong advantages for predators.

In our experiments, behavioural observations showed that *E. balteatus* females were unable to distinguish unparasitised from newly parasitised aphids and did not exhibit any preference for either prey. On the other hand, plants infested with mummified aphids and exuvia of mummies were less attractive and fewer eggs were laid close to them by hoverfly females. A key component of prey discrimination is the perception of patch quality and the adjustment of patch residence time to exploit the patch according to its relative quality. Theoretical models, principally elaborated for parasitoids, propose that a female parasitoid should allocate more time for the exploitation of patch perceived as being of good quality (Waage, 1979; McNair, 1982). Similarly, it can be expected that a predator with the ability to discriminate will adopt its searching and exploitation time according to patch profitability. Flexible residence time and giving-up time thus determine the pay-off of different patch qualities (van Alphen & Galis, 1983). In the present study, we found that *E. balteatus* females spent more time on plants infested with unparasitised or parasitised aphids in terms of acceptance and oviposition behaviors, compared to similar plants infested with mummified aphids or exuvia of mummies. A reason for this might be the ability of a generalist predator, such as *E. balteatus* to distinguish an oviposition site with high quality and to exploit the encountered patches according to their relative value. It was previously found that coccinellid *Coleomegilla maculatelengi* Timb larvae spent less time in patches containing solely old *Trichoplusia ni* eggs parasitised by *Trichogramma evanescens* Westwood, and their level of exploitation was greatly reduced, compared to similar patches containing unparasitised young *T. ni*eggs (Roger et al., 2001). The reasons behind the preference of *E. balteatus* for plantsinfested with parasitised aphids compared to those infested with mummified aphids or exuvia of mummies remain uncertain. When predators attempt to locate a prey habitat, they use odors associated with prey presence, such as those from the herbivorous prey itself (Whitman, 1988; Verheggen et al., 2007), or from prey by-products, such as feces or honeydew (Budenberg & Powell, 1992). It has been shown that parasitized *A. pisum* produce more honeydew, a carbohydrate-rich excretion, and are more likely to attract aphid predators and parasitoids that use honeydew as a kairomone (Carter & Dixon, 1984; Budenberg, 1990). Honeydew was shown to induce preference in *E. balteatus* (Sutherland et al., 2001), which may also explain thepreference of *E. balteatus* females for plants infested with parasitised aphids over plants infested by mummified aphids. In a previous study, Almohamad et al. (2007) showed that *E. balteatus* females prefer *Solanum tuberosum* L. as host plant to *Solanum nigrum* L., because apart from the aphid-released (*E*)-β-farnesene, *S. tuberosum* releases important amounts of the aphid alarm pheromone (Agelopoulos et al., 2000), whereas *S. nigrum* does not release this sesquiterpene (Schmidt et al., 2004), which is known to attract predators, such as *E. balteatus* (Francis et al., 2005). In the present study, although parasitised aphids released less alarm pheromone than unparasitised ones (FJ Verheggen, unpubl.), *E. balteatus* females did not exhibit significant preference for plants infested by unparasitised *A. pisum.*

According to Chandler (1968), selection of an adequate oviposition site by syrphid females, which lay eggs close to aphid colonies, is essential to ensure survival and development of their offspring. Some individual females of *E. balteatus* differed from others in their preferences, and at the individual level, there appeared to be life-history trade-offs in performance with these preferences (Sadeghi & Gilbert, 1999). Additionally, performance of predatory hoverfly is often affected by the aphid species (Sadeghi & Gilbert, 2000b). The quality of prey is important for survival, development, and reproduction in aphidophagous hoverflies (Almohamad et al., 2007). We clearly show that unparasitised and parasitised pea aphids are good quality food for the development and survival of second instars of *E. balteatus,* confirming the hypothesis that ovipositing insects can select sites that improve growth and survival of their offspring (Peckarsky et al., 2000). This is particularly important for insects that are unable to migrate easily from habitats poor in food, such as syrphid larvae. Our results agree with those of Brodeur (1994), who demonstrated in the laboratory that the incidence of predation by aphidophagous predators (coccinellids, syrphids, and predatory midges) was similar for parasitized and unparasitized potato aphids. Additionally, predatory midge and syrphid larvae that are aphid-specific predators may feed on recently parasitised aphids, but ignore mummified aphids (Kindlmann & Ruzicka, 1992; Harizanova & Ekbom, 1997). We indeed showed that hoverfly second instars are more reluctant to feed on mummified pea aphid. This is in agreement with Takizawa et al. (2000), who found that mummified aphids have negative effects on the growth of predatory ladybirds.

In conclusion, our results demonstrated that choice of oviposition sites by *E. balteatus* females may be affected by the presence of parasitoids in the aphids. This suggests that predator and parasitoid interactions represent an asymmetrical exploitation competition that needs to be understood to elucidate the mechanisms that shape guilds of aphidophagous insects. However, as we tested colonies that were homogeneously constituted of either unparasitised or parasitised aphids, which is unlikely to be found in nature, these conclusions should be considered carefully, and one should further investigate the ability of hoverfly females to discriminate aphid colonies that consist only partly of parasitised aphids.

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Chapter 7

Impact of syrphid female age on its reproduction

Insect female's age could be another important factor determining a forager's decision. When an organism is close to the end of its life it may be more advantageous for it to accept a poor quality oviposition site than it is for a young organism. Previous studies have indicated that young E. balteatus females show a marked hierarchical preference for particular species of aphids, while old females are less selective. Furthermore, hoverfly females do not waste their mature eggs when facing a shortage of hosts or when there is no suitable aphids. However, the influence of ageing E. balteatus individuals on reproductive efficiency is not well-documented despite its importance in biological control programs.This present chapter aims to evaluate the influence of ageing E. balteatus females on their reproduction in order to consider their optimal use in biological control programs.

Article 10

How does the age of hoverfly females affect their reproduction?

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Abstract – In the present study, we observed the effect of age of *Episyrphus balteatus* DeGeer (Diptera, Syrphidae) females on their fecundity and fertility (number and percentage of fertile eggs). Eight newborn *E. balteatus* couples were placed in separated cages (30 cm x 30 cm x 60 cm) and the number of eggs laid and the egg viability were recorded daily during 45 days. *E. balteatus* females had a pre-oviposition period of 10 days before mating happened. The fecundity increased steadily from day 11 to day 16 with an average of 30 eggs a day. During the following days (from day 23 to day 45), we found that the optimal fecundity was observed every second day and that 70.47 % of the eggs laid during the entire life of the females were fertile. We also found that the fertility decreased significantly when the females were older than 38 days. As result, the age of hoverfly females influence significantly on their reproduction, with suggesting that hoverfly females from 2 to 5 weeks old could be important agents for biological control programs.

Key words: *Episyrphus balteatus*, female hoverfly age, fecundity, fertility.

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Introduction

The first optimal foraging models (e.g. Charnov, 1976) were static and assumed that a forager's decisions depended only on extrinsic factors, i.e. patch quality, patch availability, general quality of the environment, etc. Then dynamic models were developed that stressed the importance of intrinsic factors like age, experience, energy reserves, and egg load in decision making by foragers (Mangel & Clark, 1986; Mangel, 1987, 1989). Age, through time limitation, should be an important factor determining a forager's decision; when an organism is close to the end of its life it may be more advantageous for it to accept a poor quality oviposition site than it is for a young organism (Mangel, 1989). This decline in selectivity according with age has some empirical supports. For example, aphidophagous ladybird *Adalia bipunctata* (L.) were less selective when older or when they had previously experienced poor quality patch (Frechette *et al*. 2004). As, Weisser (1994) also demonstrated that the parasitoid *Lysiphlebus cardui* Marshall becomes less selective for the age of the aphids it parasitizes when the age of parasitoid increased. However, in the field, Heimpel *et al*. (1996) found no evidence that the age affected the oviposition behaviour of the parasitoid *Aphytis aonidiae* (Mercet). The aphidophagous hoverfly *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) is one of the most efficient aphid specific predators (Tenhumberg & Poehling, 1991). The larvae of this species are predators on more than 100 species of aphids worldwide (Sadeghi & Gilbert, 2000b). Indeed; several studies show high efficacy of *E. balteatus* as biological control agent for agricultural pest aphids (Pollard, 1971; Kalshoven, 1981; Chambers & Adams, 1986; Tenhumberg & Poehling, 1995; Kreß, 1996). However, oviposition decisions of predatory hoverflies may be affected by different factors such as female age (Sadeghi & Gilbert, 2000a) and egg load (Sadeghi & Gilbert, 2000c). For examples, young *E. balteatus* and *Syrphus ribesii* (L.) females show a marked hierarchical preference for particular species of aphids, while old females are less selective (Sadeghi & Gilbert, 2000 a). Sadeghi and Gilbert, (2000 c) reported that *E. balteatus* and *S. ribesii* (L.) females do not waste their mature eggs when facing a shortage of hosts or when there is no suitable aphids. Dixon (1959) also showed that female *Eupeodes corollae* could retain mature eggs in the absence of aphids, but eventually some eggs were laid.

On the other hand, the influence of ageing predatory hoverfly *E. balteatus* on reproductive efficiency is not well-documented despite its importance in biological control programs. This study aims to evaluate the influence of ageing *E. balteatus* females on (i) their reproduction and (ii) to consider their use in biological control programs.

Materials and methods

Plants and insects – Broad beans (*Vicia faba* L.) were grown in 30 x 20 x 5 cm plastic trays filled with a mix of perlite and vermiculite (1/1) and maintained in controlled environment growth rooms (16:8 Light: Dark; 20 ± 1°C). The aphid species, *Megoura viciae* Buckton, was reared on *V. faba* plants, in an air-conditioned room set at the same conditions as above. Adult *E. balteatus* were reared in 75 x 60 x 90 cm net cages and were provided with bee-collected pollen, sugar and water. Broad beans infested with *M. viciae* were introduced into the cages for 3 h every two days to allow oviposition. Hoverfly larvae were mass-reared in aerated plastic boxes (110x140x40 mm) and were daily fed *ad libitum* with *M. viciae* as standard diet.

Experimental observations – To assess the evolution of *E. balteatus* female reproduction (fecundity and fertility) according to their age, eight couples were observed daily after hatching from pupae. Each couple (female and male) was isolated in separated cages (30x30x60 cm). In each experimental cage, water, sugar and been pollen were provided separately as food on small Petri dishes. Each day and for 45 consecutive days, a newly infested broad bean plant with *M. viciae* was offered to each female for 24 h. The plant was then removed and the number of eggs was recorded. The number of viable eggs was also observed.

Statistical analysis

Means numbers of eggs were compared using ANOVA (general linear model) and Tukey's test, conducted with Minitab® software (version 12.2, Minitab Inc, State College, PA, USA). Percentage of egg viability was transformed using the angular transformation (arcsine $\sqrt{x}$; Dagnelie, 1973), before performing analysis of variance ANOVA.

Results

The evolution of the fecundity of *E. balteatus* females according to their age is presented in Figure 23. *E. balteatus* females had a pre-reproduction phase of 10 days before mating happened. They started laying eggs 11 days after hatching. Female age had a significant influence on the number of eggs laid per day (General linear model, $F_{44,359} = 8.63$; $P < 0.001$). From day 11 until day 16, the number of eggs laid by *E. balteatus* females increased steadily with an average of 30.35 ± 8.83 eggs.

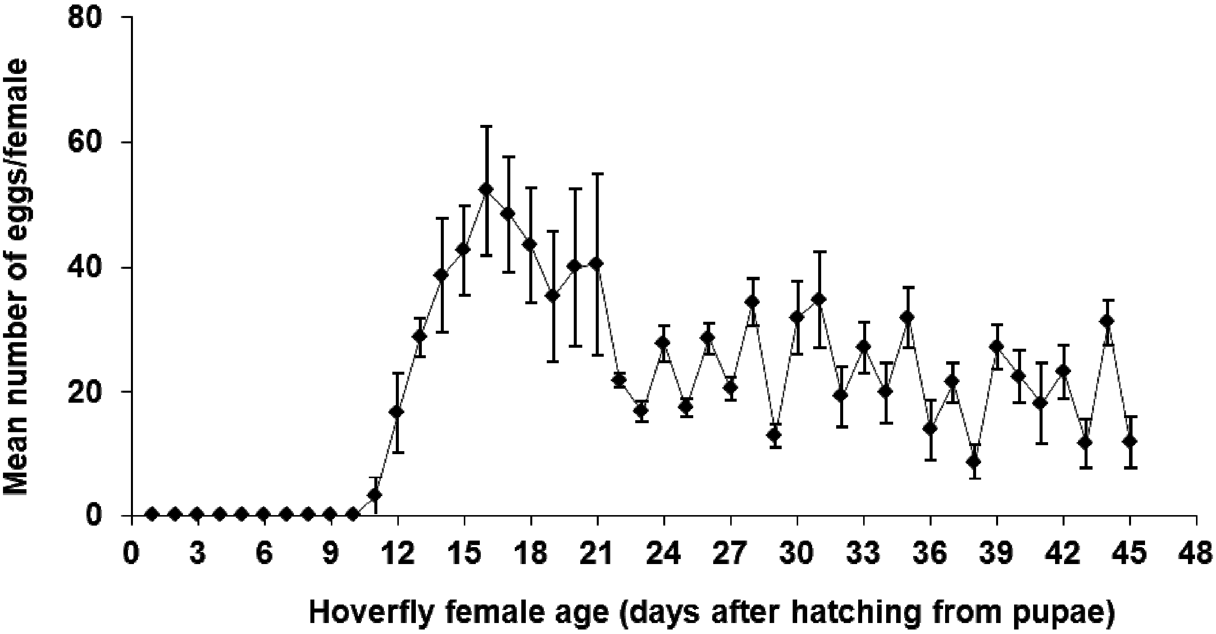

Figure 23. Evolution of the number of eggs laid per day by *E. balteatus* females as a function of age. Bars indicate the stander error.

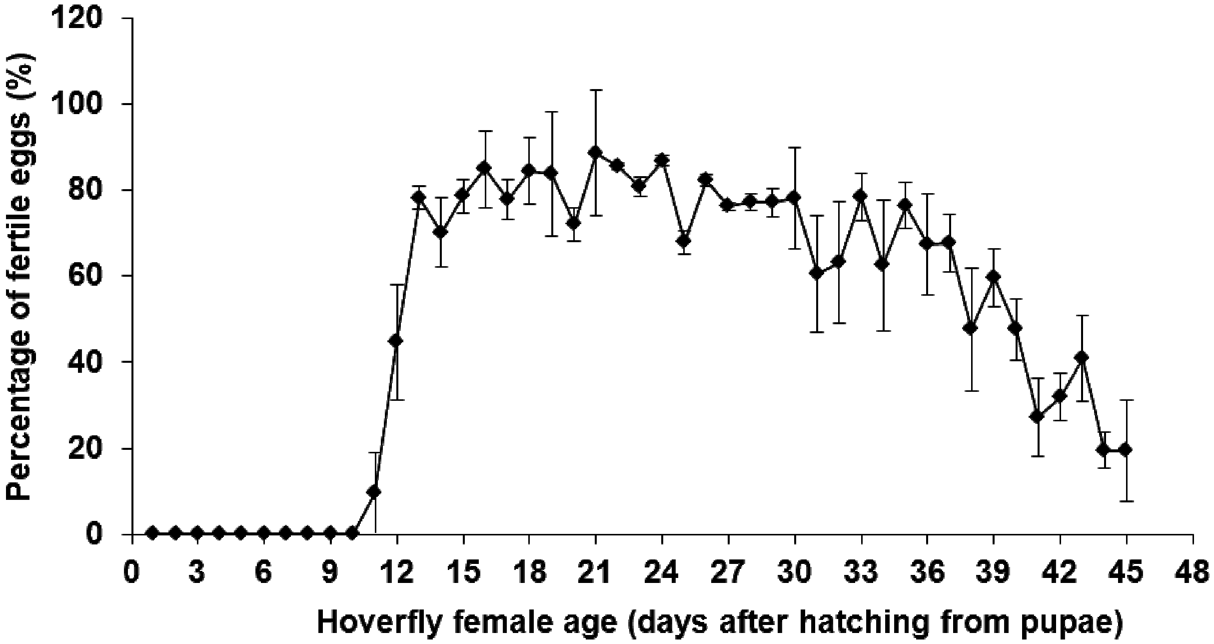

Figure 24. Percentage of fertile eggs laid per day by *E. balteatus* females as a function of age. Bars indicate the stander error.

From day 17 to day 23, an average of 35.2 ± 9.79 eggs were laid. During the following days (from 23 to 45 days old females), we found that the optimal egg laying was observed every second day. The egg viability was also observed, and the results are presented in Figure 24. The percentage of fertile eggs laid per day differ according to *E. balteatus* female age (General linear model, $F_{44,359} = 17.85$; $P < 0.001$). Most of the eggs laid per day were fertile with an average of 70.47 ± 6.51 %). Nevertheless, we found that the fertility of eggs decreased significantly when the females were older than day 38.

Discussion

These results clearly indicate that age does have a significant effect on the hoverfly female reproduction. Both lifetime fecundity and fertility were affected by the age at which females mated, both decreasing with an increase in age at mating. These effects have been previously observed in a number of many species such as, in generalist phytophage *Epiphysta postvittana* Walker (Foster & Ayers, 1996), in insect predators (Mohaghegh *et al*., 1998; Ahmad *et al*., 2004, Frechette *et al*., 2004; Omkar *et al*., 2006), and in wasp parasitoid *Trichogramma cordubensis* Vargas & Cabello (Hymenoptera: Trichogrammatidae) (Particia *et al*., 2001). However, the discussion on temporal fecundity patterns of predatory hoverfly females and the fertility of eggs according to their ages is limited. In the present study, *E. balteatus* females (from day 11 to day 38) had efficient fecundity, and the majority of eggs laid during life entite of females were fertile. Nevertheless, the fertility decreased when *E. balteatus* females wrer older than 38 days. This decline in the fertility with age of *balteatus* female remains unclear. When an insect predator is older, it may be more advantageous for it to accept a poor quality oviposition site than it is younger. According to Sadeghi & Gilbert (2000c), young aphidophagous hoverflies, *E. balteatus* and *S. ribesii* are more selective in choice of their oviposition site, while old females are less selective. The results of Chandler (1966) also showed that young *E. balteatus* females did not oviposit on uninfested plants, but they lost this discrimination when they aged, which may explain the decline in the fertility of *E. balteatus* female when they older than 38 days.

It is known that *E. balteatus* has a well-defined temporal pattern in egg production and egg-laying behaviour (Volk, 1964; Bargen, 1998, Hindayana, 2001). Our results confirmed that the number of egg-laying by *E. balteatus* females reaches its maximum evry second day. As, during the reproductive phase (from 23th day old females to 45th old females), a time period of two days between the peaks in egg production could be identified. In addition, we found that *E. balteatus* female had pre-reproductive phase of ten days, while the results of

Hindayana (2001) showed that *E. balteatus* female needed a nine days premature period before they start laying eggs. This may be due to the aphid prey type used as standard diet for *E. balteatus* larvae. In the study of Hindayana (2001), *E. balteatus* was fed with foxglove aphid, *Aulacorthum solani* (Kaltenbach) reared on fertilised cucumber.

In addition to the influence of the ageing hoverfly females on their fecundity and fertility, the food quality has been one of the main factors affecting reproductive capacity of insect predators (Evans, 1982; De Clercq & Degheele, 1992). In present study, we found that large numbers of the eggs laid by *E. balteatus* were fertile in relation to female age. An average hatching rate of 70.48 % was observed. This was slightly higher than the average hatching rate of 67.6 % observed by Hindayana (2001) and 53.6 % observed by Geusen Pfister (1987). These diffrences in *E. balteatus* reproduction according to female'age may be also due to others factors such as the food quality and /or experimental set-up. During our experiments *E. balteatus* were fed exclusively with *M. viciae* that were reared on broad bean plants, whereas Geusen- Pfister (1987) offered *E. balteatus* a mixture of *Aphis craccivora* (Koch) and *A. pisum* aphids reared on broad bean plants.

In conclusion, age of hoverfly females is an intrinsic factor influencing the reproduction capacity during the entire life of the females, suggesting that hoverflies (from 2 weeks old females to 5 weeks old females) could be an effective biocontrol tool aphid pest management.

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Chapter 8

General discussion, Conclusions and Perspectives

In central Europe, *E. balteatus* is found to be the most common hoverfly and one of the most efficient aphid-specific predators in natural agroecosystems. To promote this aphid predator as efficient biological control agent, it is of primary importance that the foraging and oviposition behaviour of females are well known. This knowledge is necessary in order to evaluate the conditions when and where it should be used as antagonist. The ability of hoverfly *E. balteatus* females to find and oviposit within the future foraging range of its progeny is however a critical determinant of potential biocontrol performance because neonate offspring have limited mobility to forage. Many potential factors influence the choice of oviposition site by hoverfly females including host plant, aphid species, prey availability, semiochemicals, presence of intra- or interspecific competitors and female's age. This PhD thesis provides with useful clues that help to better understand the behavioural mechanisms of the response to these factors, which enable *E. balteatus* females to optimise their oviposition sites and maximise their fitness. By this knowledge, we may be better able to optimise efficient use of predatory hoverfly in managing aphid pest populations.

Chapter 4 offers several suggestions for the selectivity of hoverfly female oviposition. Gravid *E. balteatus* females exhibited variations in their oviposition preference among different combinations of aphid host plant and aphid species and these differences had important consequences for the performance of their offspring (fitness). The green peach aphid *M. persicae* –infested *S. tuberosum* was the most suitable aphid-host plant combination as an oviposition site for *E. balteatus,* which ensure the completion of larval development and subsequent adult reproduction, while, *M. persicae*-infested *S. nigrum* was the least preferred aphid-host plant combination. It can be concluded that oviposition preference and fitness of *E. balteatus* could be enhanced especially when the host plant had an optimal nutritional value for the aphid prey and in consequence for the predators. This dependence of predatory hoverfly on the quality of host plant (first trophic level) is relevant in regard to the oviposition-performance theory which focuses on the host plant selection as oviposition site and its indirect effect on the aphid prey suitability for the fitness of this antagonist. These results could be taken into account in order to enhance the production and optimise the fitness of adult syrphid at the release site (i.e. in greenhouses). Similarly, our results also confirm the statement that predatory hoverflies select their oviposition site according to the infesting aphid species. The black bean aphid *A. fabae* was not as attractive for *E. balteatus* as the pea aphid *A. pisum* and the vetch aphid *M. viciae.* Moreover, the global hoverfly fitness was higher when larvae were fed with *A. pisum* or *M. viciae* as aphid prey compare to *A. fabae*.

This result was also in accordance with previous observations that demonstrated the pea aphid to be preferred among eight aphid species. However, *M. viciae* and *A. fabae* were not tested. Consequently, the second trophic level (aphids) can have a significant impact on oviposition behaviour and offspring performance of *E. balteatus*. This result would confirm the hypothesis that ovipositing insects can select sites that improve the growth and survival of their offspring, in particular for insects that are unable to migrate easily from habitats poor in food, such as syrphid larvae.

In addition, other host plant or aphid factors were also found to be involved in recognition of oviposition site by *E. balteatus* females, such as semiochemicals emitted from aphid prey itself or associated with its host plant. Our results are in accordance with previous observations that predatory hoverfly, like other natural enemies, often use odours from aphid prey or from its association with host plant when foraging for oviposition site. *E. balteatus* female was more strongly attracted to the odour of aphid-infested potato plants than to those of uninfested plants, and the volatile profiles of the two host plants are indeed different. Apart from the aphid-released (*E*)-β-farnesene, *S. tuberosum* release important amounts of the aphid alarm pheromone whereas *S. nigrum* does not release this sesquiterpene. It is well known that the sesquiterpene (*E*)-β-farnesene was found to attract *E. balteatus* females and act as foraging cue and oviposition stimuli. The response of hoverfly to this molecule and its attractive role may explain the oviposition preference of *E. balteatus* female for *S. tuberosum*. Although other host plant factors, such as colour can influence the foraging behaviour of the predatory hoverfly *E. balteatus*. It is surly that this parameter could not explain the differences we obtained, as both host plants (*S. tuberosum* and *S. nigrum*) were of similar color. It is also suggested that several aphid related factors could determine its suitability for predatory hoverfly. Among them, the size of the aphid species tested might be a factor of importance in host selection. Although similar numbers of aphids were infested broad bean plants, but whereas *A. pisum* and *M. viciae* are large aphids, *A. fabae* is slightly smaller and therefore represented less food for hoverfly offspring. On the other hand, the three tested aphid species release (*E*)-β-farnesene, but might not release similar quantities, which could be specific or size dependent. The oviposition stimulus can also come from the aphid honeydew, which varies qualitatively and quantitatively from one aphid species to another and during the season. We could conclude that the ability of *E. balteatus* to perceive chemical signals emitted from aphids or their association with host plants and utilise them to locate and select an adequate oviposition site could explain their oviposition preference to one aphid host plant or one aphid species rather than other, and offer opportunities to encourage this aphid predator into field with different crops. By using crops emitting large amounts of attractive chemicals could allow the predatory hoverfly to locate its prey at early stage and increase its efficacy in biological control of aphid populations. However, more detailed informations are required to complete our knowledge about the important role of plant or aphid semiochemicals and the oviposition behaviour of *E. balteatus* females. Further investigations could be proposed: (i) quantification the amount of (*E*)-β-farnesene and honeydew emitted by aphid species (*M. viciae*, *A. pisum* and *A. fabae*), (ii) test of behavioural impacts of these molecules toward hoverfly females, (iii) answering the question: do other molecules present in honeydew attract females or is the attractiveness due to systemically released volatiles in response to aphid species attack, (v) field evaluation experiment of these molecules as attractants for predatory hoverfly.

A part from aphid prey effects, we found that aphid colony size (number of aphids per patch) has an important influence on the selection of oviposition site by predatory hoverfly (Chapter 5). The foraging and oviposition behaviour of *E. balteatus* females was dependent on aphid colony size. Indeed, hoverfly females also seem to be able to adjust their egg number to aphid density. This result was consistent with the finding of previous studies that aphidophagous hoverflies are known to demonstrate a positive relationship between aphid colony size and oviposition. Although the number of eggs laid (oviposition rates) by predatory hoverfly females increased in response to increasing aphid colony size, the evidence is still unclear. Using leaf-disc system, which was shown to be an efficient method to evaluate hoverfly oviposition behaviour, *E. balteatus* females exhibited pronounced searching, acceptance (landing, walking, and proboscis extension) and abdominal protraction leading to egg-laying behaviour in response to increasing aphid colony size. Since syrphid larvae have limited mobility to forage, these positive behavioural responses of *E. balteatus* females (Ovipositional tactics) to increasing aphid colony sizes, suggesting an adaptive oviposition behaviour leading the emerging larvae to locate immediately sufficient food resources and optimise female's searching effort. On the other hand, the number of eggs does not only increase with the aphid density on plant or on leaf-disc system, but the *E. balteatus* females laid also more eggs in larger single colonies than in small ones. As usually, hoverfly *E. balteatus* females laid only one egg per landing, the higher numbers of eggs in large colonies must have resulted from an increased number of landings. Hence, large colonies were more attractive than smaller ones. However, it is noted that predatory hoverfly females would not lay such high numbers of eggs in a single aphid colony under natural conditions. While in laboratory experiments, females tend to lay more eggs because they are unable to disperse and no other suitable aphid colonies (i.e., aphid colony without syrphid eggs) are present. In addition, several studies have reported that females of many syrphid species (i.e. *E. balteatus*) prefer smaller aphid colonies or aphid colonies with a high proportion of early aphid instars, for oviposition and avoid heavily infested plants, especially colonies with winged aphids. It is not surprising that females avoid crowded aphid colonies, because these colonies are subjected to increased migration of the prey, rather than continued colony growth, which would ensure later hoverfly larval survival.

Others aphid colony factors (i.e. time of appearance and location) can have a significant influence on foraging of syrphid females and distribution of their eggs-laying. We found that *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. This result was confirmed by previous field observations that showed the height preference of aphid colony location for various syrphid species was already related to their aphid prey habitat preferences. Hence, syrphid species that develop 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. This could provide interesting information on the distribution of syrphid eggs in relation to aphid prey habitat and height preference of aphid colony. However further field experiment are therefore needed to better understand the impact of aphid colony location on predatory hoverfly oviposition preference.

More surprisingly, we found that the number of eggs laid by hoverfly female does not only depend on aphid quantity on the plant, but also on the presence and quantity of oviposition-eliciting substances emitted from aphid colony of different sizes such as (E)-β-farnesene. The emission of EβF was found to be released significantly in response to increasing aphid colony size in their headspace. Indeed EβF provoked a positive response in *E. balteatus* females and acts as an oviposition stimulant. This dependence suggests that this molecule could provide hoverfly females with interesting information about aphid colony size. Because the important role of EβF in the attraction and oviposition behaviour of aphidophagous syrphid, EβF could potentially be used to enhance the numbers of aphidophagous syrphid in field situations. So, syrphids may be encouraged to remain in area with presence of EβF (like that released by septum containing EβF) and lay more eggs even when aphid numbers are low. This can have a significant effect even when there are low aphid densities. Thus further field experiments would be necessary to evaluate the molecule EβF as attractant for predatory hoverfly. There are indeed other molecules such as honeydew secreted by aphid colonies that may also play important role in oviposition site selection by syrphid females, and that could provide them with interest information about aphid colony size. Therefore, intense research is necessary to understand the infochemical role of honeydew in predatory hoverfly response to aphid colony size.

In chapter 6, we have highlighted that *E. balteatus* oviposition behaviour was strongly modified by the presence of intra-or interspesific competitors when foraging for oviposition site. Females avoided aphid colonies in which conspecific larvae were already present. Indeed, the oviposition deterring stimulus was also active in presence of conspecific larval tracks. As previously discussed, the choice of aphid patch as oviposition site has important impact on the offspring fitness. Thus the oviposition decision made by hoverfly female would favor aphid patch by avoiding eggs-laying in sites where competitors threaten the survival of its offspring. Apart from intraspecific effects, cannibalism of eggs and first instars larvae appears to be an important regulation factor in aphid predator performance under rearing or application conditions, and it has been well documented in aphidophagous hoverflies such as *E. balteatus*. Therefore, the tendency of syrphid females to oviposit less in aphid colonies in which conspecific larvae or their tracks were present might serves to reduce the risk of egg cannibalism by developed larval stages. This is one reason that may explain this oviposition avoidance shown by *E. balteatus* females in response to conspecific larvae and their tracks. On the other hand, this repellency of conspecific larvae or their tracks to hoverfly oviposition may be mediated by different chemical cues. Previous observations have demonstrated that aphidophagous predators such as ladybirds are sensitive to volatiles signaling the previous or actual presence of conspecific larval tracks. For example, the extracts of *Adalia bipunctata* larval tracks involve a mixture of alkanes that have multiple semiochemical functions in coccinellids including mate-recognition, defense and oviposition deterrence. Similarly, *E. balteatus* females were deterred from ovipositing in response to the chemical cues present in the odor extracts of conspecific larval tracks. It has been also reported that aphid predator females possibly monitor in the assessing an aphid prey patch both the concentration of the oviposition-deterring substances and the stimulatory cues associated with aphids and rely on the concentration of these stimuli to assess the risk of their eggs being eaten. This behaviour was clearly shown by predatory hoverfly, where the *E. balteatus* females reduced significantly their oviposition rates in response to different amounts of oviposition-deterring substances left by conspecific larvae in aphid patch. It could be concluded that hoverfly female response in this way to semiochemical cues could optimise females searching effort by reducing the time needed to assess aphid patch quality and consequently increase the probability of detecting suitable oviposition site.

Similar oviposition avoidance was also shown by predatory hoverfly in response to the presence of heterospecific competitors. We found that *E. balteatus* females oviposited less often in aphid colonies in which larval tracks of coccinellid *H. axyridis* were present. Field and laboratory observations demonstrated that *E. balteatus* usually coexists with *H. axyridis* in many habitats, and syrphid eggs were vulnerable to predation by *H. axyridis* larvae. Thus, the oviposition avoidance shown by *E. balteatus* females in response to the presence of *H. axyridis* larval tracks could reflect the low risk of predation and optimise conditions for their offspring performance, confirming the optimal oviposition theory. We also demonstrated that the foraging behaviour hoverfly female was modified by the presence of parasitoids *Aphidius ervi* in aphid colonies. *E. balteatus* females were unable to distinguish unparasitised from newly parasitised aphids and did not exhibit any preference for either prey. They were less attractive and fewer eggs were laid in response to the presence of mummified aphids and their exuvia on plants. Indeed we clearly show that unparasitised and parasitised pea aphids are good quality food for the development and survival of second instars of *E. balteatus*, but hoverfly second instars are more reluctant to feed on mummified pea aphid. Considering the cost in energy and time of moving from one patch to another, this behaviour shown by hoverfly females could be considered as adaptive that allows to ensuring the larval survival and optimises the female's searching effort. A key component of prey discrimination is the perception of patch quality and the adjustment of patch residence time to exploit the patch according to its relative quality. Theoretical models, principally elaborated for parasitoids, propose that a female parasitoid should allocate more time for the exploitation of patch perceived as being of good quality. Similarly, it can be expected that a predator with the ability to discriminate will adopt its searching and exploitation time according to patch profitability. Flexible residence time and giving-up time thus determine the pay-off of different patch qualities. These result support our finding that *E. balteatus* females spent more time on plants infested with unparasitised or parasitised aphids in terms of acceptance and oviposition behaviours, compared to similar plants with mummified aphids or exuvia of mummies present. A reason for this might be the ability of a generalist predator, such as *E. balteatus* to distinguish an oviposition site with high quality and to exploit the encountered patches according to their relative value. Additionally, oviposition avoidance of parasitised aphid colonies by syrphid predator *E. balteatus* could be mediated by different infochemical cues. It has been shown that parasitised *A. pisum* produce more honeydew, a carbohydrate-rich excretion, and are more likely to attract aphid predators and parasitoids that use honeydew as a kairomone. Honeydew was shown to induce preference in *E. balteatus,* which may also explain the preference of *E. balteatus* females for plants infested with parasitised aphids over plants infested by mummified aphids. Although alarm pheromone was to be attractive for *E. balteatus* females, and its amount released from parasitised aphids was less than unparasitised ones (FJ Verheggen, unpubl.), *E. balteatus* females did not exhibit significant preference for plants infested by unparasitised *A. pisum.* These results suggest that the effects of both the syrphid predator and aphid parasitoid *A. ervi*, could be complementary used in biological control of aphid *A. pisum* populations, however for successful biological control with two aphid natural enemies, *A. ervi* females are preferred to be released at two weeks before the releasing of *E. balteatus* in order to avoid the intraguild predation that could be occurred among them. Thus further investigation is needed to evaluate the efficacy of two aphid natural enemies in biological control of aphids in natural habitats (i.e. greenhouses or field).

Finally, age of *E. balteatus* females was found to influence the reproduction capacity during the entire life of the females, with suggestion that younger *E. balteatus* female (2 to 5 weeks old) could have potential to be an effective biocontrol agent of aphids because of its higher reproductive efficiency. Moreover, the number of fertile eggs was decreased when the *E. balteatus* females were older than 38 day old. This decline may be explained because older *E. balteatus* females are less selective in choice of favorable oviposition site. It could be concluded that to obtain an optimal exploitation of *E. balteatus* in biological control efforts, younger females of *E. balteatus* are preferred to be released.

Beside the ability of hoverfly *E. balteatus* females to evolved behavioural mechanisms of response to some of biological and ecological factors, that enabling them to be selective in choice of oviposition site in way that ensure their offspring performance and optimise their searching efforts in evaluation of aphid patch quality, intense research on the infochemical role of substances induced by plant or aphids (i.e. honeydew, EβF) and evaluations of their attractive effects toward hoverfly *E. balteatus* in natural conditions (i.e. field and greenhouses), would lead to more perspectives for successful biological control of aphid populations with this aphid predator. In addition, predatory hoverfly is not the only antagonist that can be used e.g. in greenhouses, thus it is also important to consider intraguild interaction among antagonists. This knowledge needs further investigation for evaluation the potential of predatory hoverfly as biological control agent of aphids with others aphid natural enemies (predators and parasitoids), and the important role of infochamical cues the mediate these interactions. Finally, these studies will enhance greatly our understanding about the chemical ecology of aphidophagous hoverflies and their intraguild interactions