

**The role of resource subsidies in enhancing biological control
of aphids by hoverflies (Diptera: Syrphidae)**

A thesis
submitted in partial fulfilment
of the Degree of Doctor of Philosophy
at
Lincoln University

by
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Lincoln University

2007

*All animals, except man, know that the principal
business of life is to enjoy it.*

Samuel Butler



Dedicated to Clément and Roman Janzac

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

The role of resource subsidies in enhancing biological control of aphids by hoverflies (Diptera: Syrphidae)

By E. A. Laubertie

Abstract

In this thesis, experiments were conducted in the laboratory and the field to determine whether the provision of floral resources to hoverflies could enhance the biological control of aphids. The overall aim was to clarify hoverfly behaviour and ecology in an agroecosystem in order to understand the potential of these insects for biocontrol under a conservation biological control (CBC) regime.

A preliminary experiment in New Zealand compared the effect of different coloured water-traps on catches of the hoverflies *Melanostoma fasciatum* (Macquart) and *Melangyna novaezelandiae* (Macquart). Significantly more individuals were caught in completely yellow traps than in traps with green outer walls and yellow inner walls or in completely green traps. This suggested that if a measure of hoverfly numbers relating to a particular distance along a transect is required, consideration should be given to the ability of hoverflies to detect yellow traps from a distance. The use of traps that are green outside would more accurately reflect the local abundance of hoverflies, as the insect would be likely to see the yellow stimulus only when above or close to the trap. Also, the addition of rose water significantly increased the number of *M. fasciatum* caught.

From a suite of flowering plants chosen for their ability in other studies to increase hoverfly visit frequencies, laboratory experiments were conducted in France to determine the plant's effectiveness at enhancing *Episyrphus balteatus* (De Geer) 'fitness', and to evaluate whether adult feeding on flowers was related to performance. Phacelia (*Phacelia tanacetifolia* Bentham cv. Balo), followed by buckwheat (*Fagopyrum esculentum* Moench cv. Katowase) and coriander (*Coriandrum sativum* L.) gave the optimal reproductive potential of female *E. balteatus*. There was no correlation between pollen and nectar consumption, and there was no discernible positive

correlation between the quantity of pollen ingested and the resulting female performance.

Phacelia and buckwheat were then studied as resource subsidies in the field in New Zealand. The effect of incorporating phacelia or buckwheat in the margins of 5 m x 5 m broccoli plots was tested for hoverfly activity and floral 'preferences'. Hoverflies which had fed on phacelia and buckwheat pollen were found up to 17.5 m from the floral strips and females of *M. fasciatum* and *M. novaezelandiae* consumed more phacelia pollen than that of buckwheat in the field. These results support the choice of phacelia as an ideal floral resource subsidy in crops for enhanced biological control by these New Zealand species.

The need for studying hoverfly movement in a large-scale field experiment was apparent from the field studies, so the next experiment was carried out in a field 450 × 270 m and flies were marked via their ingestion of the pollen of phacelia. The focus was on the proportion of flies having consumed the pollen. Although large quantities of pollen were found in some hoverfly guts, most did not contain phacelia pollen and very few were captured at 50 m from phacelia, compared with numbers at the border of the floral strip. A possible explanation was that hoverflies feed on a large variety of pollen species, reducing the relative attraction of phacelia flowers. Another possibility was that hoverflies dispersed from the phacelia away from the crop. Also, pollen digestion rates are likely to be a factor.

Finally, a series of experiments was conducted in the field and laboratory to study hoverfly efficacy through oviposition and larval behaviour. In field experiments, female *M. fasciatum* and *M. novaezelandiae* laid more eggs where buckwheat patches were larger; however higher oviposition rates did not lead to improved aphid population suppression. In greenhouse experiments, larvae of *E. balteatus* could initiate a decline in aphid numbers at the predator: prey ratio 1: 8.3, however this control did not persist. Experiments in the laboratory showed that hoverfly larvae became more active and left the system while aphid numbers declined or numbers of larvae increased. This behaviour was caused by two factors: hunger and avoidance of conspecific larvae. Further experiments showed that the avoidance of conspecifics was caused by mutual interference rather than cannibalism.

The results of this work highlight the importance of hoverfly dispersal ability. Given the observations of foraging behaviour of females and mutual interference observed between larvae, and the lack of success in CBC by hoverflies in experiments at the crop scale, it is essential to assess the impact of insect predators and parasitoids at a landscape scale.

Keywords

Hoverflies, *Melanostoma fasciatum*, *Melangyna novaezelandiae*, *Episyrphus balteatus*, aphids, selective resource subsidies, conservation biological control, fitness, hoverfly movement, oviposition, foraging behaviour, mutual interference.

Acknowledgements

I thank my supervisor Steve Wratten and co-supervisor Jean-Louis Hemptinne for their constant support, help and friendship. For your hard work commenting in all my manuscripts and, Steve, your patience with our emails. Merci!

Many thanks to all the people that helped me in the field, especially Emilie Lemaire, Guillaume Caillot, Chris Lowe, Tom Birkett, Martin Guer, Susie Clearwater, Susan Blyth, Dane Riding, Ashley and David Pontin. And to my girls Shona and Joe Randell, I had a really good time with you sampling those hundreds of lettuces!! A special thanks to Don Heffer from the Field Service Centre, Lincoln University, for field logistics. I also thank Mark Malcom and Tim Chamberlain whom allowed me to set up my experiment in their carrot field. Thanks to Brent Richards for his help growing the plants in the greenhouse in my first year!

Huge thanks to André Maureau for his time and help growing the plants in the French greenhouse, building those beautiful cages, taking care of my syrphids while I was in New Zealand, and his terrible sense of humour!

For his help and comments I thank my great friend Michel Canard.

Thanks to Roger Creswell and Lynn Clucas for their time and help getting the atomic absorption spectrometer (AA) to read rubidium.

Thanks to Alison Lister and Richard Sedcole for their advices on statistical analyses.

Big thanks to Guy Jalut for helping me identifying pollens.

I would also like to specially thank Dianne O'Connor and Dianne Fyfe for their time and help.

To all the staff and students from the group, especially Blas Lavandero, Patrik Kerhli (the big brothers!), Samantha Scarratt, Muriel Gevrey, Mauricio Urrutia, Dharme Madurappulige, Kay Sakata, thank you very much. It has been a great pleasure.

Special thanks to the French team: Alexandra Magro, Naima Boughenou, Aurélie Ferrer, Xavier Martini, Jérôme Garcia, Nicolas Bastin, Jean-François Garrigues, and of course Aurélie Navarre, Sylvie and Nadine.

I would also like to acknowledge FRST granting me the opportunity to study in New Zealand, without which this project would never have been.

Last, but not least, I would like to thank my friends and family for providing continual encouragements and reminding me that at the end it would all be worth it. And a special thank to my boy Clément for having followed me in New Zealand and supporting me through the whole PhD process.

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Chapter 1

General introduction

Faced with a strong demand for production increases, agriculture has suffered massive intensification and a heavy use of pesticides. However, chemical control of pests, weeds and diseases has limitations: target pest resurgence, secondary pest outbreaks, environmental pollution, hazards to human health and, because of their usual broad spectrum of activity, pesticides are often toxic to non-target beneficial and endangered species. At the same time, improper or excessive use of pesticides has led to the development of resistance in pests to the extent that some insects are difficult or impossible to control, even at increased dosages (Georghiou, 1986; Palumbi, 2001). Current worldwide pesticide use has increased to more than 3 billion kilograms per annum but pest problems have not declined as expected. More than 40 % of all world food production is still lost to insect pests (15 %), plant pathogens (13 %) and weeds (12 %) (Pimentel, 2004).

Biological control can be an extremely important alternative to widespread pesticide use as well as a significant component of sustainable agriculture. The value of biological control as an 'ecosystem service' (Costanza *et al.*, 1997) has been given at an annual value of US \$ 417 billion by Costanza *et al.* (1997). Biological control of arthropods involves the use of predators, parasitoids and pathogens to reduce pest densities below the economic damage threshold (Pickett & Bugg, 1998). It may be implemented in a number of ways. Classical biological control involves the introduction of new species into an area to control an exotic pest. Augmentative biological control comprises the mass rearing and inoculative or inundative release of natural enemies. Conservation biological control (CBC) modifies the environment to protect and enhance enemy populations to increase their effectiveness at managing the target/pest organism (Debach & Rosen, 1991).

The present research project focuses on agroecosystem manipulation through an appropriate introduction of floral resources to attract and enhance the effectiveness of one natural enemy group, the hoverflies.

In practice, CBC is effected by either a careful use of pesticides (better targeting in time and space, narrow spectrum of efficacy, reduced rates, etc.) or by habitat manipulation to enhance natural enemy fitness and effectiveness (Barbosa, 1998; Eilenberg *et al.*, 2001; Gurr *et al.*, 2004b). This enhancement may be achieved by providing the appropriate diversity of resources essential to natural enemy persistence and to the stability of their populations. Resources such as nectar, pollen, alternative prey and hosts, physical refugia and lekking sites that natural enemies need to feed, reproduce and thrive, are important (van Emden, 1990; White *et al.*, 1995; Hickman & Wratten, 1996; Baggen & Gurr, 1998; Landis *et al.*, 2000; Gurr *et al.*, 2004b; Nicholls & Altieri, 2004; Heimpel & Jervis, 2005). Providing the appropriate diversity means that the flowering plants introduced will benefit the natural enemy but not the pest (Speight, 1983; van Emden, 1990; Gurr *et al.*, 2000a; Gurr *et al.*, 2003), or at least favour the natural enemy more than the pest (e.g. Baggen & Gurr, 1998; Scarrat *et al.*, 2004).

As previously mentioned, CBC involves the use of habitat manipulation techniques; however, the two approaches remain quite separate (Fig. 1.1). Habitat manipulation employs techniques of cultural control such as crop diversification, which is common to CBC, the difference being that the plant protection that results from habitat manipulation is a more ‘bottom-up’ (first trophic level) mediated approach. This stems from the ‘resource concentration’ hypothesis (Root, 1973), in which phytophagous populations are determined by a lower trophic level; pest suppression occurs as a result of non natural-enemy effects and it includes such processes as host-plant finding by phytophagous. This hypothesis argues that concentrated areas of host plants are easier to find and colonize. The presence of plants distantly related to the crop plots has direct negative effects on the ability of the insect pest to find its host plant, ‘diluting’ the visual plant stimulus and potentially masking other stimuli such as odours (Root, 1973; Gurr *et al.*, 2000b).

CBC generates ‘top-down’ (third trophic level) effects, referred also as the ‘enemies’ hypothesis (Root, 1973). This hypothesis argues that pest numbers are reduced in more diverse systems because the activity of natural enemies is enhanced by providing key ecological resources (Gurr *et al.*, 2000b; Landis *et al.*, 2000).

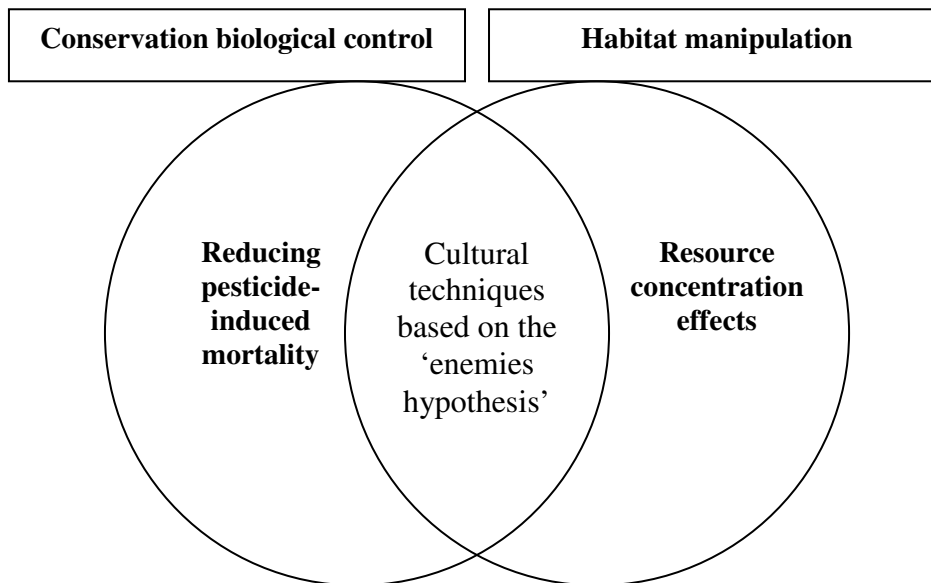


Figure 1.1. Comparing and contrasting habitat manipulation and conservation biological control approaches to pest management. From Gurr *et al.* (2000a).

CBC of existing natural enemies has long been a rather neglected form of biological control but interest in this field has increased markedly during the last decade (Gurr *et al.*, 2004a). In part this has been motivated by the relatively low success rate of classical biological control of arthropods by arthropods over the years (Gurr & Wratten, 2000) and the risk that it causes undesired side-effects on non-target organisms (Louda *et al.*, 2003). Moreover, the full potential of natural enemies has rarely been realised in conventional agriculture because most crops are monocultures and are lacking of extra-floral resources; in addition they are treated with pesticides. Also, relatively little attention has been given to the ecological requirements of the agent involved, resulting in poor availability or provision of key ecological resources. Recently, increased research has been focused on the ecology of natural enemies and their prey/hosts, in order to enhance the effectiveness of biological control agents (Wratten & van Emden, 1995; Gurr *et al.*, 1998, 2003; Gurr & Wratten, 1999, 2000; Landis *et al.*, 2000; Kean *et al.*, 2003; Gurr *et al.*, 2004a; Tylianakis *et al.*, 2004; Zehnder *et al.*, 2006).

In this thesis, the focus is on hoverflies which are generalist beneficial insects. The larvae of many species are predators of aphids while adults feed on nectar and pollen.

Syrphids represent a useful model for research in conservation biological control, as evidence from the literature indicates that the provision of added floral resources may enhance local hoverfly populations (Molthan & Ruppert, 1988; Sengonça & Frings, 1988; Lövei *et al.*, 1992b; MacLeod, 1992; White *et al.*, 1995; Morris & Li, 2000; Day, pers. comm.). Studies have been conducted in the field to determine the ‘preferences’ of hoverflies for different flower species (Cowgill *et al.*, 1993a; Hickman *et al.*, 1995; Colley & Luna, 2000) thereby establishing the optimal plant species.

Although floral resource subsidies increase hoverfly visit frequencies, it does not follow that more eggs will be laid near floral resources because syrphids are highly mobile and able to cover great distances. Chandler (1968a) found no difference in oviposition in small replicated plots of Brussels sprouts between those with buckets of flowers added and those without. Densities of the bean aphid *Aphis fabae* (Scopoli) in sugar beet plots with phacelia (*Phacelia tanacetifolia* Benth) were reduced by syrphids compared with populations in control plots (Sengonça & Frings, 1988), but the density of eggs and larvae was also higher in control plots where aphid density was highest. However, White *et al.* (1995) showed that sowing phacelia strips around the borders of a cabbage crop significantly increased the populations of *Melanostoma fasciatum* (Macquart) and *Melangyna novaezelandiae* (Macquart) and decreased the number of aphids. However they worked on 17.5 × 45 m plots and this did not give any information on hoverfly dispersal and their efficacy on a whole field. In fact they found the highest numbers of hoverflies in the traps closest to the phacelia strip border, and numbers of syrphid eggs did not differ between treatments. Day (pers. comm.) used yellow plastic model flowers (with no trapping aim) to assess the impact on hoverfly oviposition in their vicinity, and found that oviposition was significantly higher near the yellow models than in control position (brown model flowers). Hickman and Wratten (1996) found that more adult syrphids were caught in plots bordered with phacelia than in control plots, indicating that the influence of phacelia extended to at least 180 m from the border strip. Moreover more eggs were found in winter wheat fields bordered with phacelia than in control, and fewer aphids were present in these fields. However, the absolute difference in aphid density was small and there was no evidence that syrphid predation (with phacelia) had an economic benefit. Despite the above examples, the question of whether or not the resource subsidies can increase hoverfly fitness and efficacy in the field remains largely unanswered. Although floral resource subsidies enhance hoverfly abundance, it does not

follow that the flies remain in the crop itself, and lay enough eggs so that the larvae performed a sufficient control to avoid using pesticides.

Various species of hoverflies occur as larvae in agricultural and horticultural crops. In New Zealand farmland, hoverflies are represented by only two common species: *M. fasciatum* and *M. novaezelandiae* (Miller, 1921; Early, 1984). Although both these species are abundant throughout the country, *M. fasciatum* has been thought to be more common, especially in the North Island (Miller, 1921). In Europe, for instance, *Episyrphus balteatus* (DeGeer) and *Metasyrphus corollae* (F.) are the most abundant species on arable land (Dean, 1982). Their voracious aphidophagous larvae have been identified as being economically important, with the potential to halt aphid population growth (Chambers & Adams, 1986). The present study focuses on *E. balteatus*, *M. fasciatum* and *M. novaezelandiae*, by working in the northern and southern hemispheres. As larvae of those species are generalist feeders, the system studied are not much important and crops used are broccoli (*Brassica oleracea* L. var. *Italica*), carrots (*Daucus carota*, L.), lettuces (*Lactuca sativa* L. cv. Target) and broad beans (*Vicia fabae*, L.).

Thesis aims

The overall aim is to clarify hoverfly behaviour and ecology in an agroecosystem in order to understand the potential of this insect for biocontrol when conservation biological control is practised. Although there are clear benefits of providing hoverflies with floral resource subsidies, further work is needed to select resources, if there are any, which fulfil the nutritional requirements of the flies. It could be ecologically and economically more interesting to leave fallow strips colonized by weeds. Research is also necessary to determine the spatial scale over which pollen and nectar sources affect hoverfly dynamics, the optimal densities and spacings for planting flowering plants.

Goals are:

1. To determine if the traps commonly used are efficient to measure local populations of hoverflies.
2. To determine the effects of a range of flower species on hoverfly 'fitness', in terms of longevity and fecundity, and rank them under laboratory conditions.
3. To compare the use of the best candidate flowers selected previously and hoverfly preference in the field.
4. To study *M. fasciatum* and *M. novaezealandiae* dispersal from selected floral resources into the crop in a large-scale experiment, and to address the following question: do hoverflies remain in floral resource subsidies area, or do they feed but then disperse to find other suitable floral resources?
5. To determine whether predation rates increase in the presence of floral resource subsidies and lead to a reduction in pest populations in the field. If not, to understand why.

Chapter 2

What are hoverflies?

Hoverflies (Diptera: Syrphidae) can be distinguished from all other dipterans by the presence of a ‘false vein’ (*vena spuria*) in the middle of the wing, between the radius and the media (Coe, 1953). Adult hoverflies are highly active, usually brightly coloured insects and have an aptitude for hovering. According to Vockeroth and Thompson (1987), the family Syrphidae comprises three subfamilies (Syrphinae, Milesiinae and Microdontinae), 180 genera, and about 6000 described species. Hoverflies can be found almost anywhere: they are present on every continent apart from Antarctica, and belong to one of the most species-rich families of dipterans.

The adults feed mostly on pollen and from flowers but can also feed from the leaf surface, where aphids have produced honeydew. The larvae display an impressive range of feeding habits, which can be considered in four categories:

- species that feed on plant tissue and plant products, such as sap
- species that scavenge on or filter decaying matter, usually in water
- species that live in the nests of social insects (bees, wasps and ants)
- carnivorous, the normal food of which is aphids.

From the point of view of pest biological control, the most important species are those which feed on aphids as larvae. Most aphidophagous species are found in two tribes of the subfamily Syrphinae: Syrphini and Melanostomini, both of world-wide distribution.

The role of non-host resources for hoverflies: importance of pollen and nectar

Hoverflies are synovigenic insects. Females hatch with an immature reproductive system: they require the proteins and amino acids of pollen to mature the ovaries and sustain egg production (Schneider, 1948; Chambers, 1988). The preoviposition period lasts for about a week (Schneider, 1948; Stürken, 1964; Geusen-Pfister, 1987). If they

do not experience food shortages, females lay eggs continuously from the start of oviposition until death. (Geusen-Pfister, 1987; Branquart & Hemptinne, 2000a). Nectar, the other principal food, contains only trace amounts of these substances, and provides energy. Once mature, male hoverflies probably need rather little in the way of proteins and amino-acids, while the females still require large amounts for maturing successive batches of eggs (Gilbert, 1993).

Despite the importance of adult nutrition in the ecology of hoverflies, little is known about the effects of pollen and nectar on fecundity and particularly on longevity. It is assumed that the nutritive value of pollen lies in its proteins and amino-acids. Stürken (1964) found a drastic reduction in the number of eggs laid by *M. corollae* when any single amino acid except cystine was omitted from an artificial diet. The amount of nectar and pollen available to adult hoverflies can have a significant effect on egg load of the females (Scholz & Poehling, 2000). Most of the work done on hoverflies and floral resources has concerned adult syrphid responses to flower strips and on hoverfly floral 'preferences'.

The dependence of adult hoverflies on pollen and nectar raises the potential for enhancing biological control by syrphid larvae *via* the provision of flowers around or across fields. This may be especially valuable in modern agroecosystems where agronomic practices have removed many of the flowering weeds and non-weed vegetation (Boatman, 1989). The addition of particular flowering plant species can increase natural enemy visit frequencies and enhance biological control of aphids (Harwood *et al.*, 1992; Lövei *et al.*, 1992b; Harwood *et al.*, 1994; White *et al.*, 1995; Hickman & Wratten, 1996; Nicholls *et al.*, 2001; Zehnder *et al.*, 2006). The increase in beneficial insect efficacy observed is due to the combined effects of increased survivorship, fecundity, retention and immigration (Altieri & Whitcomb, 1979; Altieri & Letourneau, 1982). While the nutrients from pollen and nectar support metabolism and gamete development for many insect predators and parasitoids, flowers also provide mating sites and alternative prey (Leius, 1967; Altieri & Whitcomb, 1979). Syrphids were observed feeding on a range of flower species (Cowgill *et al.*, 1993a), but within the range, some species were used selectively (MacLeod, 1992; Lövei *et al.*, 1992a; Cowgill *et al.*, 1993a) with floral features such as colour, depth of corolla tube, and nutritional value of pollen being important factors in determining preferences (Gilbert,

1981; MacLeod, 1992). A few flowering plants have been experimentally evaluated as insectary plants including phacelia (*Phacelia tanacetifolia*), coriander (*Coriandrum sativum* L.) and buckwheat (*Fagopyrum esculentum* Moench) (MacLeod, 1992; Lövei *et al.*, 1992a, 1993; Hickman *et al.*, 1995; Colley & Luna, 2000; Wratten *et al.*, 2003). Yellow and white flowers are also often included in lists of attractive species because these colours elicit feeding in hoverflies (Cowgill, 1989). Umbelliferous (Apiaceae) flowers such as coriander and fennel have short corollae, facilitating nectar accessibility (Gilbert, 1981), also making them good insectary plants. *Episyrphus balteatus* was seen taking pollen of mainly Apiaceae and Compositae, compared with that of anemophilous grasses, possibly because pollen of the former species are more nutritious (Gilbert, 1981). Cowgill (1990) produced a host plant feeding ‘preference’ index for *E. balteatus*. In field boundaries in June in the northern hemisphere, creeping thistle (*Cirsium arvense* L.) and sowthistle (*Sonchus oleraceus* L.) were the most preferred, followed by red deadnettle (*Lamium purpureum* L.) and white campion (*Silene latifolia* Poiret). Moreover, Branquart and Hemptinne (2000b) reported *E. balteatus* as a generalist feeder, dominant at all sites they inspected, irrespective of the type of habitat. It was found feeding mostly on Asteraceae.

Holloway (1976) showed that small, sparsely haired hoverflies with a short proboscis, such as *M. fasciatum* ingested at least 99 % anemophilous pollen from Plantaginaceae or Gramineae (Poaceae) and that larger, more hairy hoverflies with pollen-collecting hairs and elongated mouthparts ingested pollen almost exclusively from nectar-bearing flowers. *Melangyna novaezelandiae*, an intermediate stage with a small number of curly-tipped pollen-collecting hairs and an elongated proboscis fed on entomophilous pollen from Liliaceae. Wratten *et al.* (1995) reported that although 40-50 % of all individuals of *M. novaezelandiae* had been feeding from Gramineae, *Taraxacum*-type (Compositae) was the most frequently occurring pollen in the gut of *M. fasciatum* and *M. novaezelandiae*. Moreover, they found no Plantaginaceae pollen in *M. fasciatum*; these observations reflected the plant species available in the study area. Hickman *et al.* (1995) found that Plantaginaceae, Apiaceae and Gramineae pollen was consumed in large quantities by gravid *M. fasciatum* females.

Biological control by hoverfly larvae

Larvae of *M. novaezelandiae* and *M. fasciatum* prey on a large number of aphid species (Rohitha *et al.*, 1985) and also account for a large proportion of arthropod predation of early-instar Lepidoptera such as those of the white butterfly, *Artogeia rapae* (L.), and the diamondback moth, *Plutella xylostella* (L.) (Miller, 1918, 1921; Valentine, 1967; Ashby & Pottinger, 1974). Syrphid larvae can prevent population increases (Chambers & Adams, 1986) or actually cause a decrease in aphid populations (Chambers & Sunderland, 1982; Chambers *et al.*, 1986; Sengonça & Frings, 1988). For example, Tenhumberg and Poehling (1991) found that an 80-90 % reduction in cereal aphid populations occurred six days after second-instar *E. balteatus* larvae were released into field cages, where the ratio of prey to predator was as high as 245:1. However the reasons for success or failure were never analysed in detail.

Hoverfly ecology and constraints

Hoverflies are attractive potential conservation biological control agents because of their intimate relations with flowers, and with aphids. By definition, an efficient biological control agent must give a sufficient aggregative response to cause a decline in the pest population which has a higher growth rate than the growth rate or attack rate of the predator (Beddington *et al.*, 1978). In general, predator efficacy depends on its consumption rate. Moreover it is also important to know about female fecundity and oviposition, as efficacy of hoverfly larvae depends on how the adults lay eggs among aphid colonies. Insect oviposition behaviour is a vital component in understanding a great many elements of insect evolutionary biology, and hoverfly ecology is submitted to several constraints:

Aphid colonies are ephemeral food sources in space and time, of which development time is hardly longer than the development time from egg to adult stage of the predator. Sometimes they are hugely abundant, but even abundant colonies can disappear over very short time scale. It requires special adaptations to be able to take advantage of them.

The resource is used by other natural enemies: intraspecific predators, which can imply risks of cannibalism, or by other species associated with a risk of interspecific predation.

Hoverfly larvae have rather limited dispersal ability (Chandler, 1969; Sadeghi & Gilbert, 2000; Gilbert, 2005). The selection of an adequate oviposition site by the syrphid females, that is laying eggs close to an aphid colony, is essential to secure the survival and fast development of their offspring.

While many authors have reported that positive density-dependent oviposition occurs in *E. balteatus* (Chandler, 1968b; Geusen-Pfister, 1987; Tenhumberg & Poehling, 1991; Bargaen *et al.*, 1998; Scholz & Poehling, 2000), several studies suggest that female syrphids 'prefer' smaller or younger aphid colonies for oviposition (Kan & Sasakawa, 1986; Kan, 1988a, 1988b, 1989; Hemptinne *et al.*, 1993). They avoid plants with large or ageing aphid colonies, especially those with alate adults (Kan, 1988a, 1988b), which is termed the 'buy-futures' ovipositional tactic (Sutherland *et al.*, 2001a). This tactic ensures that eggs are not wasted on aphid colonies which are due to disperse in the near future. The former studies consider this density dependence as adaptative behaviour, as it secures larval survival and economizes on female's energy spent on reproduction (Bargaen *et al.*, 1998). As usually only one or a few eggs are laid per landing, the higher number of eggs in large colonies must have resulted from an increasing number of landings. Moreover, there is also evidence that hoverflies exhibit a low aphid density 'oviposition threshold'; Ambrosino *et al.* (2007) pointed out that hoverflies avoided broccoli plants without aphids, as well as plants with less than 50 aphids.

However, the suitability of an oviposition site does not depend only on the number and species of aphids present at the time of oviposition. Other predators may have consumed aphids by the time the larvae hatch and may even feed on syrphid eggs; therefore females lay only one or a few eggs in an aphid colony, then fly off and avoid aphid colonies with syrphid eggs, as long as aphid colonies without eggs were present (Kan, 1988a; Hindayana *et al.*, 1999; Scholz & Poehling, 2000). Larvae of *E. balteatus* attack syrphid eggs and smaller syrphid larvae (Branquart *et al.*, 1997): therefore the avoidance of aphid colonies with syrphid eggs would be an adaptative behaviour for the syrphid female (Scholz & Poehling, 2000).

The egg laying behaviour of hoverflies remains complex to understand, and the experiments quoted above illustrate this complexity. While some authors consider density dependence in hoverfly oviposition to be an adaptative behaviour, evolutionist arguments relate egg production to optimal foraging theory, with aphid colonies which are already exploited being avoided as are those that will not last long enough to support the development of hoverfly larvae (Hemptinne *et al.*, 1993).

If female hoverflies avoid plants with large or ageing aphid colonies, and/or already exploited, they might not be efficient biological control agents. Studies cited in these first two chapters are not sufficient to be sure of any CBC action success. Although floral resource subsidies attract many hoverflies, they don't remain in the crop itself, and lay enough eggs so that the larvae fulfilled a sufficient control to avoid using pesticides.

These observations prompted us to set up an experiment where the proportion of floral resource subsidies added to the crop would be so large that the crop area would become derisory. Exaggerating this way the CBC action, we aim to determine whether female fecundity is high enough to control aphid colonies. Moreover we will study larva behaviour in a system getting more and more reduced, from bean plants in greenhouse to Petri dishes in laboratory in order to observe their predatory efficacy and reasons which would justify their behaviour.

Chapter 3

The role of odour and visual cues in the pan-trap catching of hoverflies¹

Hoverflies are among the most abundant natural enemies of aphids in agricultural and horticultural crops. Their larvae can reduce aphid populations (Chambers & Sunderland, 1982; Chambers *et al.*, 1986) and therefore these insects have potential as biological control agents. The larvae of many species are predatory but adults feed on pollen and nectar, so many members of the family exhibit 'life-history omnivory' (Polis & Strong, 1996). The dependence of adult hoverflies on pollen and nectar raises the potential for enhancing biological control by syrphid larvae via the provision of flowers around or within fields.

In New Zealand farmland, hoverflies are represented by only two common predatory species: the native *M. fasciatum* and the endemic *M. novaezelandiae* (Miller, 1921; Early, 1984). Larvae of both species consume large numbers of aphids (Rohitha *et al.*, 1985) and also account for a large proportion of arthropod predation on early-instar lepidopteran larvae, such as those of the white butterfly, *Artogeia rapae*, and the diamondback moth, *Plutella xylostella* (Miller, 1918, 1921; Valentine, 1967; Ashby & Pottinger, 1974). Therefore, the conservation of adult natural enemies by the provision of floral resources at the field (Molthan & Ruppert, 1988) or landscape (Thies & Tschamtker, 1999; Thies *et al.*, 2003) scale can have benefits in the management of several pest species. Understanding predators' phenology is a vital component of conservation biological control. The most frequent technique involves the provision of appropriate nectar to enhance the longevity and/or fecundity of beneficial insects (Gurr *et al.*, 2005). Flowering phenology can be manipulated, largely by varying drilling times, but it needs to be matched to that of the target pest and its key natural enemies. Hence the need for appropriate insect trapping techniques.

An understanding of the dynamics of the movement of adult hoverflies, and of their ability to disperse from floral resources into the crop, is needed to ensure the most

¹ Status: published as Elsa Laubertie, Steve Wratten and Richard Sedcole (2006). The role of odour and visual cues in the pan-trap catching of hoverflies (Diptera: Syrphidae). *Annals of Applied Biology*, **148**, 173-178.

efficient deployment of floral resources to enhance biological control (Lavandero *et al.*, 2004a). This dispersal is usually assessed by trapping.

Coloured traps (water, sticky, etc.) are frequently used to attract and catch insects. They can be useful in monitoring populations of predatory and other beneficial insects, such as in 'classical' or 'conservation' biological control research. They are also frequently used in the evaluation of the recovery of insect populations after disturbance (e.g., pesticide use, crop harvest, habitat fragmentation, etc.). In such insect-dispersal studies, marking of the organisms is common (Lavandero *et al.*, 2004a).

Evidence suggests that aphidophagous hoverflies have a strong preference for yellow (e.g., Kevan & Baker, 1983; Finch, 1992; Hoback *et al.*, 1999; Sutherland *et al.*, 1999; Day, pers. comm.), and yellow water traps are often used to attract adult hoverflies in order to assess population size (Smith, 1976; Lövei *et al.*, 1992b; Harwood *et al.*, 1994; White *et al.*, 1995; Irvin *et al.*, 1999). Wratten *et al.* (1995) showed that yellow traps were the most efficient at trapping *M. novaezelandiae*, whereas for *M. fasciatum* there were no differences between yellow, white and blue traps; there were however significantly fewer adult hoverflies in green traps for both species.

For insect community studies to be of value, the sampling methods must produce samples representative of the community or taxon selected for investigation. However, the fact that traps may be visible to the insects over a considerable distance can influence the interpretation of catch data. This difficulty may arise if traps along a transect can attract insects from some or all of the other transect positions. To investigate a more efficient coloured water trap for sampling 'local' populations of insects, different designs of traps have been compared in the present work, in a flat, vegetation-free area. The absence of vegetation was necessary to ensure that the outer walls of the traps were visible to the insects.

However, depending on the type of the study, it may be necessary to capture as many insects as possible, for statistical purposes for example. Yellow is highly attractive to hoverflies, but odour is also likely to be involved in hoverflies' detection of flowers (Molleman *et al.*, 1997). The influence of floral odour (rose water in this case) on hoverfly attraction was examined here.

This study compared the effect of different coloured traps on attraction and catch of hoverflies. The hypothesis was that traps with yellow external and internal walls attract hoverflies from a distance, while traps which are green outside and yellow inside catch fewer flies, because only those from above or near the trap can see the yellow stimulus. A subsidiary hypothesis is that rose water enhances hoverfly capture rates, as it has been observed to be the case for some parasitoids (Hoddle, pers. communication).

Materials and methods

The traps were arranged 3 m apart in a randomized complete block design in a 30 × 50 m area of bare soil, in 'Inversen Field' at Lincoln University. There were four treatments: (1) inner and outer containers yellow; (2) outer container green and inner container yellow; (3) both containers green; (4) both containers yellow plus rose water (10 ml of a commercially available rose water composed of deionised water, ethanol, bronopol, rose perfume and diethyl phthalate). Details of the formulation are commercially sensitive and are not available. The four treatments were replicated 10 times each. The traps used were two-litre plastic containers (170 × 170 × 85 mm). One was used as a trap base and anchored to the ground with a bent metal rod through its centre. A second was inserted into the base and was three-quarters filled with water, 0.5 ml of detergent (dish-washing liquid) and mono-propylene glycol as a preservative. Yellow-trap reflectance peaked at 500 nm and remained at 60 %, while green peaked at 520 nm but did not show invisible reflectance (Wratten *et al.*, 1995). As the number of hoverflies captured per trap was low, the trap contents were emptied and the traps were resampled weekly between 15 January and 4 March 2004. This ensured sufficient hoverflies for analyses. Trap contents were decanted through a 2 mm mesh sieve and transferred to 70 % ethanol in plastic 50 ml vials. In the laboratory, adult hoverflies were extracted individually using forceps and all other insects discarded. For each treatment, hoverflies were identified to species, sex (eyes touching dorsally: males; eyes not touching: females) and were counted.

The number of insects caught for the categories: species, sex, trap colour and block over the period of the experiment were re-expressed as the mean number caught per trap for each block. Thus the analysis was based on a randomised complete block with a trap by sex by species factorial treatment structure. A square-root transformation resulted in better residual plots from an analysis of variance (GenStat, Lawes Agricultural Trust, 2002) than the original data and this was used to determine significances of main effects and interactions (Table 3.2). Because rose water had an aromatic effect, separate from the colour effect of the other traps, the variance due to the differences between traps was partitioned into ‘rose water vs plain water’ and ‘between plain water’. This partition and the

interaction of this and other effects are indicated by indented entries in Table 3.2. Where the ANOVA test indicated significant differences among groups, these were further explored using an LSD test.

Results

Hoverfly temporal pattern of catches

TABLE 3.1

Number (proportions) of hoverflies of each species caught in different colours of trap between 15 January and 4 March 2004.

Treatment	Yellow + Rose water	Yellow	Yellow/Green	Green
<i>No of replicates</i>	40	80	80	80
Mf ♀	24 (44)	14 (32)	12 (52)	3 (43)
Mf ♂	30 (56)	30 (68)	11 (48)	4 (57)
Mf total	54	44	23	7
Mn ♀	17 (94)	28 (78)	14 (78)	3 (100)
Mn ♂	1 (6)	8 (22)	4 (22)	0 (0)
Mn total	18	36	18	3

Mf, *Melanostoma fasciatum*; Mn, *Melangyna novaezelandiae*.

Over the entire trapping period of 56 days, 203 hoverflies were caught. In total, 128 (63 %) individuals of *M. fasciatum* and 75 (37 %) *M. novaezelandiae* were trapped (Table 3.1).

From 15 January to 12 February 2004, catches of *M. fasciatum* were low, with a daily maximum of 1 individual per 10 traps (Fig. 3.1). From 12 February, catches increased to a daily maximum of 7 individuals per 10 traps. Catches of *M. novaezelandiae* reached a peak of 3.4 on 15 January 2004. After this date, catches were relatively low and varied from 0.4 to 2.3. This phenological information is needed to help in the design and deployment of conservation biological control practices (see Introduction).

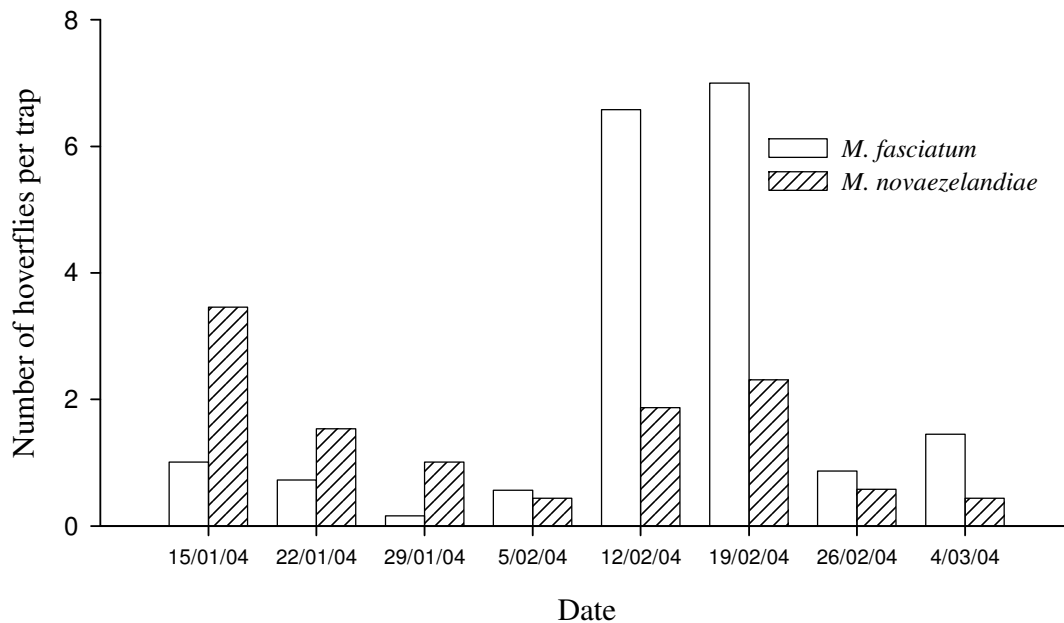


Figure 3.1. Total numbers of the hoverflies *M. fasciatum* and *M. novaezelandiae* (per 10 traps/day) captured between January 15 and March 4, 2004.

Trap colour

For each species, significantly more individuals were caught in completely yellow traps than in yellow and green or in completely green traps ($F = 24.12$; $df = 2, 135$; $P < 0.001$) (Fig. 3.2). There were highly significant ($F = 20.72$; $df = 1, 135$; $P < 0.001$) effects due to traps and species, and also due to sex ($F = 9.62$; $df = 1, 135$; $P < 0.002$). Highly significant interactions existed between sex and species ($F = 29.55$; $df = 1, 135$; $P < 0.001$), and traps and species ($F = 9.78, 135$; $df = 3$; $P < 0.001$), but not between sex and traps. There was evidence of a second-order interaction between traps, species and sex ($F = 3.44$; $df = 3, 135$; $P < 0.02$). The summary data (Table 2) indicated that the traps with rose water had a marked effect, compared with the other traps, so the trap effect was partitioned into rose water and water-only traps. The analysis then showed

that the trap by species interaction was almost entirely due to the rose-water trap by species ($F = 29.33$; $df = 1, 135$; $P < 0.001$), and the water-only traps showed no significant interaction with species. In fact, both male and female *M. fasciatum* were highly attracted by the rose water while for *M. novaezelandiae* only the females showed an attraction. Fewer numbers of males were captured in rose water traps than in water-only traps (Fig. 3.3).

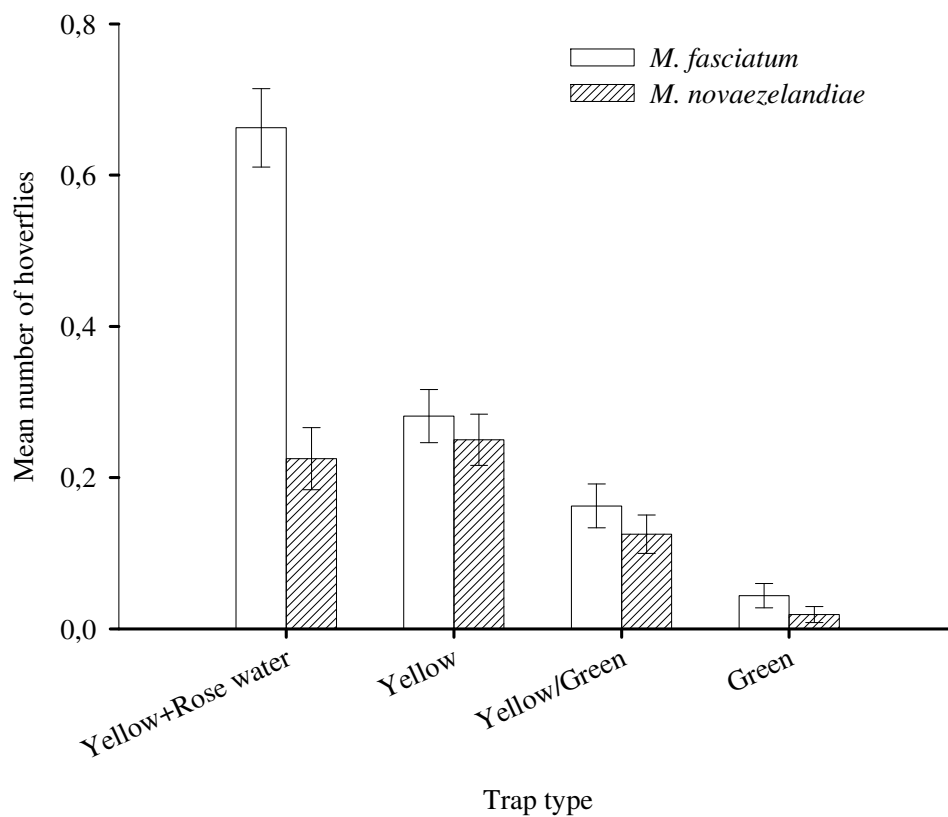


Figure 3.2. Mean (\pm SE) number of *M. fasciatum* and *M. novaezelandiae* caught per week per trap of each colour.

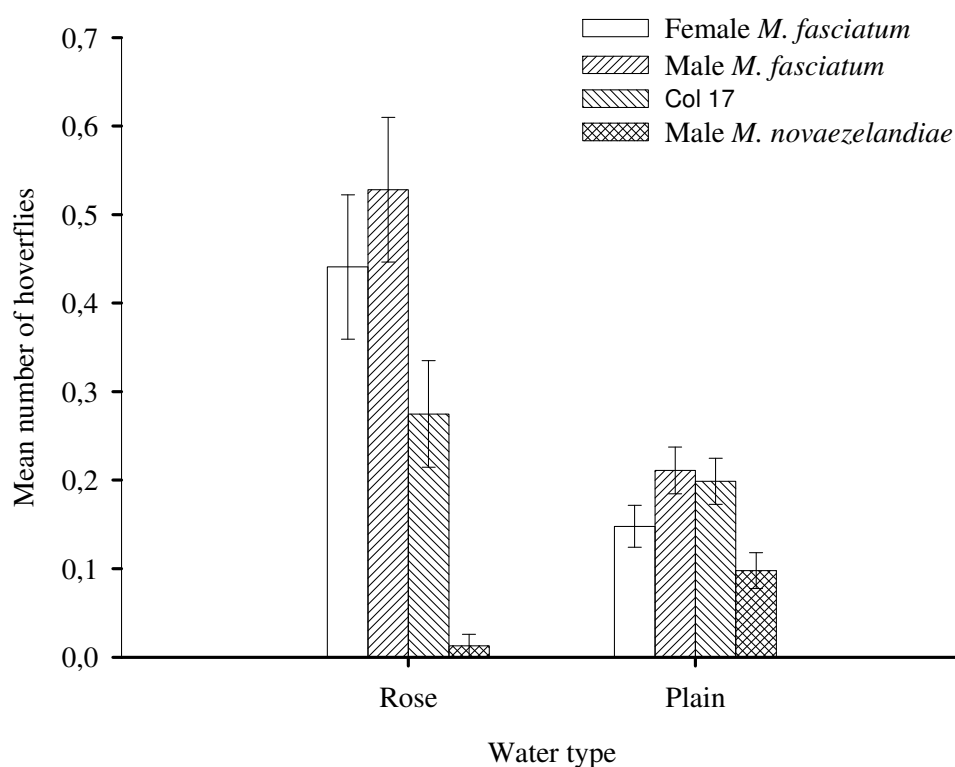


Figure 3.3. Mean (\pm SE) number of male and female *M. fasciatum* and *M. novaezelandiae* trapped in rose- and plain-water traps.

TABLE 3.2

Analysis of variance of mean number of hoverflies caught per trap (square root transformation). Indentation indicates partitioning of the main effect or interaction.

Source of variance	df	Mean square	F-ratio	Significance
Block	9	0.0665		
Species	1	0.9638	20.72	<0.001
Sex	1	0.4473	9.62	0.002
Species x Sex	1	1.3741	29.55	<0.001
Trap colour	3	1.6995	36.54	<0.001
Rose water vs Water only	1	2.8553	61.40	<0.001
Between plain	2	1.1216	24.12	<0.001
Species x Trap colour	3	0.4548	9.78	<0.001
Species x Rose water vs Water only	1	1.3640	29.33	<0.001
Species x Between Water only	2	0.0003	0.01	0.994
Sex x Trap colour	3	0.0970	2.09	0.105
Sex x Rose water vs Water only	1	0.2342	5.04	0.026
Sex x Between Water only	2	0.0284	0.61	0.545
Species x Sex x Trap colour	3	0.1599	3.44	0.019
Species x Sex x Rose water vs Water only	1	0.2086	4.49	0.036
Species x Sex x Between Water only	2	0.1355	2.91	0.058
Residual	135	0.0465		

Discussion

The hypothesis that yellow traps with yellow walls capture larger numbers of hoverflies than do yellow traps with green walls was supported by the results. This implies that the yellow-walled traps were visible to more hoverflies than were the green-walled ones, including flies remote from the trap location. Also, rose water enhanced hoverfly capture rates.

Hoverflies use vision to select a floral feeding site (Kan, 1988a, 1988b; Haslett, 1989a; Lunau, 1993; Sutherland *et al.*, 1999), so it can be assumed that colour is an important cue in helping these foraging insects to find resources. However, the results of the rose-water treatment suggest that olfactory signals also play a role in hoverfly orientation, at least for *M. fasciatum*. Visual attraction of hoverflies is well studied (see above), but there is little published work on the role of odour in hoverfly attraction. In this context, Molleman *et al.* (1997) investigated the possibility of trapping predators of pear psyllids with methyl salicylate as an attractant. They found that the syrphids *Episyrphus auricollis* (Meigen), *Metasyrphus luniger* (Meigen), *Metasyrphus corollae* and the ‘*Syrphus ribesii* (L.)’ complex were attracted to this compound. In contrast, *Episyrphus balteatus* did not show a response.

The spatial scale of this study is rather small, and it can be suggested that the distance of 3 m between traps is not enough to avoid the problem of interference between trap catches. However the aim of this study was not to evaluate the actual hoverfly population ‘density’, but to compare different trap types and results do show significant differences between treatments at the spatial scale used. The trends are clear, assuming both species studied exhibit a significant preference for yellow traps and *M. fasciatum* was even more attracted by the rose-water odour.

The results of the current experiment support those of Hickman *et al.* (2001), who suggested that yellow water traps are unsatisfactory to assess populations of hoverflies because they are perceived as food sources and are more likely to attract hungry hoverflies than satiated ones. Moreover, they compete with real food sources and consequently a higher proportion of the actual hoverfly population may be trapped in food-deprived control areas than in those where floral resources are provided

(MacLeod, 1992). In addition, the number of hoverflies caught in pan traps can be influenced by other important factors, such as the fact that yellow-walled traps attract hoverflies from an unknown distance, including individuals from outside the experimental area. Experiments in which hoverflies are marked automatically (e.g., via rubidium chloride (Graham *et al.*, 1978; Freeman Long *et al.*, 1998) or pollen (Wratten *et al.*, 2003) would be appropriate for investigating the distances over which yellow traps of different designs, and those with rose water attract these insects. More accurate evaluations of the population effects of adding floral resources to agro-ecosystems are needed (Gurr *et al.*, 2004a) and the refinements to trapping techniques analysed here can contribute to those improvements.

At present the use of traps which are green outside and yellow inside is recommended. Such traps seem to be more appropriate to reflect the local abundance of insects and if higher trap catches are needed for statistical purposes, rose water may be added.

Chapter 4

Ranking of flowering plant species in relation to adult hoverfly 'fitness'

The addition of flowering plants has been widely shown to increase visit frequencies of syrphids to the flowers and in some cases to enhance biological control of pests in adjacent fields (Molthan & Ruppert, 1988; Sengonça & Frings, 1988; Lövei *et al.*, 1992b; Harwood *et al.*, 1992, 1994; White *et al.*, 1995; Hickman & Wratten, 1996; Nicholls *et al.*, 2001). Understanding the use of floral resources by adult syrphids is important in an applied context because of the potential it may have for an efficient manipulation of agroecosystems (MacLeod, 1992; Cowgill *et al.*, 1993a).

Syrphids use floral resources selectively (Gilbert, 1981; Lövei *et al.*, 1992a; MacLeod, 1992; Cowgill *et al.*, 1993a; Lunau & Wacht, 1994) although this can vary among species and it is independent of floral availability (Haslett, 1989b; Cowgill *et al.*, 1993a). Hoverfly flower visiting behaviour has been analysed in observational studies (Gilbert, 1981; Lövei *et al.*, 1992a; Cowgill *et al.*, 1993a; Colley & Luna, 2000; Powell *et al.*, 2004) and by the presence of pollen in the diet (Haslett, 1989b). Floral features such as colour, depth of corolla tube, and nutritional value of pollen are important factors in determining preferences (Gilbert, 1981; Haslett, 1989b; MacLeod, 1992). However, a combination of factors such as kairomones, availability of flower species, arrangement in/around the crop, shelter and presence of competitors or prey all might influence landing and feeding behaviour by adult hoverflies.

Observations of the pattern of flower visit by syrphids have revealed inter-specific differences in the type of flower visited and the food taken. Analysis of the gut content of hoverflies has shown that some species exhibit a substantial degree of pollen selectivity while others show a more generalist approach to foraging (Haslett, 1989b). Although many weed species are used by hoverflies (Holloway, 1976; Gilbert, 1981; Cowgill, 1990; Cowgill *et al.*, 1993a; Wratten *et al.*, 1995; Hickman *et al.*, 1995; Branquart & Hemptinne, 2000b), studies in New Zealand demonstrate that a few flowering plants have been experimentally evaluated as insectary plants, including phacelia (*Phacelia tanacetifolia*), coriander (*Coriandrum sativum*) and buckwheat

(*Fagopyrum esculentum*) (Lövei *et al.*, 1992a, 1993; Hickman *et al.*, 1995; Wratten *et al.*, 2003). Similar results were obtained in Europe (MacLeod, 1992) and in the United States (Colley & Luna, 2000). MacLeod (1992) compared five crops in the UK: buckwheat, coriander, sunflower (*Helianthus annuus* L.), borage (*Echium lycopis* L.) and marigold (*Calendula officinalis* L.) and found coriander and buckwheat to be the favoured flowers on any date. The relative visit frequencies of hoverflies on 11 species of insectary plants in garden plots was assessed in Oregon (Colley & Luna, 2000) and coriander was the favoured species. However, alyssum (*Lobularia maritima* L. Desvaux), buckwheat, phacelia and mustard (*Brassica juncea* L.) were ranked highly on the basis of feeding visit frequencies. Marigold was relatively under visited. Although hoverflies exhibited a high degree of selectivity, this ‘preference’ was clearly influenced by what other flowers were available. One plant became the most preferred species when another highly visited species stopped flowering.

These observations highlight the point that hoverfly floral preferences fluctuate. Courtney *et al.* (1989) formalised some ideas on host plant choice by individual insects into a model which allows an animal to change diet during lifetime. They stressed that the probability that an insect will feed or oviposit on a particular host will depend on the ‘acceptability’ of the host to the insect, and this will be affected by innate tendencies and past experience. Thus at each host encounter, it is as though a number of factors are put in the balance. If the net effect is positive, the host is accepted, if negative it is rejected: however, thresholds for acceptance of hosts vary. Factors affecting acceptability should either be intrinsic to the particular host or dependent on the state of the insect. Acceptance will depend on the balance of those factors. Factors such as age, egg load, hunger of the insect, availability of prey or an assessment of whether mixtures are better than single species (Pontin *et al.*, 2006) will affect acceptance of all potential hosts.

The fact that hoverflies feed on a range of flower species (flowers recorded as beneficial insectary plants as well as weeds) has implications for the production of guidelines for the management of field margins. For example, MacLeod (1999) compared the abundance of *E. balteatus* along two strips of an arable field margin with contrasting floral richness: a control strip with no additional floral resources to those naturally present and a margin strip supplemented with eight species of flowering plants.

Episyrphus balteatus visited the rich floral strip more often, and its rate of dispersal was slower from the florally rich strip than from the control. However, *E. balteatus* was significantly more abundant at the rich strip on only three dates of the seven sampled, and differences between the rates of dispersal were not significant.

Flowers such as phacelia, buckwheat and coriander are often included in beneficial insectary plantings; however is it possible to introduce a floral resource area large enough to modify hoverfly perception of the landscape? Ecologically and economically, is it not more interesting to leave fallow strips colonised by arable weeds? This would, however, require the relative value of the weed species to be identified.

The choice of flowers selected as beneficial insectary plants has been made from both a comparison of the time hoverflies spent visiting included flower species, and an analysis of the gut content for the presence of pollen in the diet (MacLeod, 1992; Lövei *et al.*, 1992a; Cowgill *et al.*, 1993a; Colley & Luna, 2000; Ambrosino *et al.*, 2006). However, effects of those flower species on hoverfly fitness have never been studied yet.

The study reported here had two aims. The first was to compare several potential selective food plant species for their effectiveness at enhancing *E. balteatus* 'fitness', in terms of longevity and fecundity of the adults (see Kean *et al.*, 2003). It was decided to compare flowers which have commonly been included in conservation biocontrol involving hoverflies: phacelia, buckwheat, coriander, mustard and alyssum. Moreover, one species supposed to be less useful, namely marigold, was also included in this selection in order to know if apparent attractiveness was related to effectiveness. The second aim was to evaluate whether hoverfly feeding was related to performance, meaning whether female hoverflies which had the highest fitness had consumed more pollen and/or nectar.

Materials and Methods

No-choice experiment

Six flower species were compared: buckwheat (*F. esculentum* cv. Katowase), phacelia (*P. tanacetifolia* cv. Balo), marigold (*C. officinalis*), alyssum (*L. maritima*), coriander (*C. sativum*) and charlock mustard, *Sinapis arvensis* (L.). The selection was based on the results of Colley and Luna (2000).

Flowering plant species were grown from seeds and maintained in a glasshouse at the Ecole Nationale de Formation Agronomique (ENFA), Auzeville, France; all plant species were sown weekly to make sure there were flowers throughout the experiment. As the sowing-to-flowering time of the species was different, each plant species was tested as it bloomed, avoiding the problem of blooming synchronization and the need for a large number of insects. For this, the candidate plant was transported to the laboratory and placed in a mesh cage (40 × 75 × 50 cm) at 21°C and a 16 h photoperiod. Broad bean plants (*Vicia faba*) were prepared in a greenhouse: 3 plants were grown in each 13-cm diameter pot and then infested at the third leaf stage with 2 ml of *Acyrtosiphon pisum* Harris of mixed instars. This corresponded to about 200 aphids. Aphids were left for 24 h, so the aphids could settle. Hoverflies were obtained from a stock culture. In each treatment a pair of newly emerged adult *E. balteatus* and an infested broad bean plant were introduced. To minimize variability between insects, males and females used for any given plant were used within 24 h of eclosion. For each plant tested, twelve replicates were established. The experiment was conducted between 6 July 2005 and 8 September 2006. Humidity was not measured, but cages were sprayed twice a day to keep them moist. Cages were checked daily for hoverfly survival, and the bean plants were removed then replaced, so the number of eggs laid on the plants was counted. Males were used only for mating; when one died it was immediately replaced.

The following components of performance were measured for each treatment: adult length of life, number of eggs laid and egg laying life span (the number of days during which females laid eggs). The resulting data were used to determine survival, mean longevity and reproductive potential of *E. balteatus* on different flower species. The net

reproductive rate R_o , defined as the mean number of female progeny produced by a single female during its mean life span, was calculated from these data according to the method of Morales-Ramos & Cate (1992) and Doury & Rojas-Rousse (1994).

$$R_o = \sum_{x=0}^w l_x \cdot m_x$$

where l_x is the probability of survival from the day the adult emerge to the beginning of the class x , m_x is the mean numbers of eggs laid by female of age class x and w is the last class of age. Although one cannot infer about the state of equilibrium of the cohorts of syrphids presented with six species of dicotyledons in this experiment, R_o appeared as the most practical index of fitness.

Differences in the proportion of females which were successful in laying eggs between treatments were analyzed using χ^2 . Mean longevity, mean egg laying life span and mean fecundity of female *E. balteatus* were compared between treatments using a one-way analysis of variance (ANOVA) and multiple comparisons were performed using Tukey's HSD test.

Correlation between pollen/nectar feeding and performance of female *E. balteatus*

Groups of two or three newly emerging females were released in cages for 24 h with one of the six flower species selected above. A cotton wool pad that had been soaked with water was placed in all treatments. After 24 h, insects were placed in a 1.5 ml plastic tube and frozen at -80°C . Female hoverflies were removed from the tubes and placed individually on glass slides to be dissected. Head and thorax were removed carefully, and then one of the following tests was done:

Anthrone test: the presence of fructose can be analysed using the cold anthrone test (Walsh & Garms, 1980; Stewart & Kline, 1999). To realise this, 1 ml of anthrone reagent was added and the abdominal contents dissected out and extruded on to the slide. A 50×20 mm cover slip was applied to spread the preparation and the slide was then viewed under a $400 \times$ compound microscope. The presence or absence of sugar feeding in all samples was determined after an hour. If a colour reaction occurred (anthrone solution changed from yellow to dark green), then it was deemed that fructose was present in the body of *E. balteatus* and it was considered to be nectar fed. Because

the cold anthrone test does not detect the low levels of fructose present in the unfed insect (Stephun & Wäckers, 2004), fructose detection by this test would indicate that feeding had occurred (Olson *et al.*, 2000; Lee *et al.*, 2004). After an hour the positive samples turned dark green so the presence or absence of nectar feeding could be recorded.

Safranin: two drops of 0.0025 % aqueous safranin was added and the abdominal contents dissected out and extruded on to the slide (Hickman *et al.*, 1995; Wratten *et al.*, 1995; Wratten *et al.*, 2003). The safranin and abdominal contents were stirred gently to separate and spread any pollen present. A 50 × 20 mm cover slip was applied to spread the preparation and the slide was then viewed under a 400 × compound microscope. Total number of pollen grains present was estimated on a semi-quantitative scale by classifying pollen number as accurately as possible, into one of the 6 frequency classes (0 = 0 grain, 1 = 1-10 grains, 2 = 11-100 grains, 3 = 101-500, 4 = 501- 2000, 5 > 2000).

For each flower species and each test, 10 females were analysed. Proportion of female *E. balteatus* having fed on pollen in one of the 6 frequency classes and proportion of females having fed on nectar were compared between the different flower species using χ^2 analysis.

Results

No-choice experiment

TABLE 4.1

The influence of different flower species on various performance measures of adult female E. balteatus. Flower species are in order of the net reproductive rate achieved by females feeding upon them (bottom row). Treatments labelled with the same letter are not significantly different (ANOVA, Tukey's test: $\alpha = 0.05$).

	<i>Phacelia</i>	<i>Buckwheat</i>	<i>Coriander</i>	<i>Alyssum</i>	<i>Mustard</i>	<i>Marigold</i>
No females that laid eggs	7	7	11	4	3	2
% females successful in laying eggs	58	58	92	33	25	16.6
For the females successful in laying eggs only:						
Mean longevity (<i>days</i>) \pm SE	30 \pm 6.4 AB	45.3 \pm 4.6 B	23.7 \pm 1.4 AB	31.8 \pm 10.2 AB	16 \pm 3.5 AB	31 \pm 7 A
Mean egg laying life (<i>days</i>) \pm SE	15.6 \pm 4 AB	27.5 \pm 5.6 B	13 \pm 1.6 AB	17.25 \pm 11 AB	2.7 \pm 0.9 A	10.5 \pm 8.5 A
Mean oviposition rate (<i>eggs/day</i>) \pm SE	41.3 \pm 9.3 B	16.91 \pm 2.9 AB	19.5 \pm 3.3 AB	16.26 \pm 4.2 AB	46.6 \pm 21.3 AB	7.25 \pm 1.8 A
Mean no eggs laid \pm SE	600.9 \pm 192.5	498.7 \pm 121.5	242.2 \pm 42.2	192.5 \pm 87.2	90 \pm 14.2	61.5 \pm 43.5
Mean 1 st egg-laying (<i>day</i>) \pm SE	14 th	15 th	12 th	15 th	11 th	20 th
Net reproduction rate R_o	349.37	290.43	220.88	63.79	27	10.16

The flower species significantly affected the proportion of female *E. balteatus* which did not die during the preoviposition period (Schneider, 1948; Stürken, 1964; Geusen-Pfister, 1987) ($\chi^2 = 18.77$; $df = 5$; $P > 0.01$) with coriander increasing it to the greatest extent, then buckwheat and phacelia increased it equally (Table 4.1). Very low numbers of female hoverflies which had fed on pollen and nectar from mustard and marigold lived long enough to be able to lay eggs. For the syrphids which had fed on alyssum, although many females lived for a long time, a low proportion was successful in laying eggs.

Adult longevity was significantly different between treatments ($F = 3.122$; $df = 5, 64$; $P = 0.014$; Table 4.1) with buckwheat increasing it to the greatest extent, but differed significantly only from marigold. Following buckwheat, longevity was increased by phacelia, coriander and alyssum. While some female *E. balteatus* were still recorded

alive after 60 days when provided with buckwheat, phacelia and alyssum, the mortality rate was quite high, particularly in the alyssum treatment; females which fed on coriander did not live more than 34 days; however, after 25 days, more than 50 % of them were still alive (Table 4.2).

Egg-laying life and oviposition rate were significantly affected by the flower species but not in the same way. Egg-laying life was greatly influenced by buckwheat ($F = 3.418$; $df = 5, 64$; $P = 0.008$; Table 4.1), which was significantly different from marigold and mustard treatments. On the other way, phacelia increased female oviposition rate to a greater extent ($F = 2.727$; $df = 5, 64$; $P = 0.027$; Tables 4.1 & 4.2) but differed significantly only from marigold. Oviposition rate was also high when females fed on mustard pollen and nectar, however, given their longevity (16 days) and egg-laying life (less than 3 days), the lifetime fecundity (mean number of eggs laid over their life) was relatively low. The lifetime fecundity was significantly affected by treatment (Table 4.1) but was not related to female length of life. Hoverflies laid the highest number of eggs (600.9 ± 192.5) when fed on phacelia, while syrphids which fed on buckwheat laid 498.7 ± 121.5 eggs.

The net reproductive rate summarizes the performance of female *E. balteatus* when provided with different flower species. It differed among flower treatments, with females fed on phacelia and buckwheat having the highest reproductive rate (Tables 4.1 & 4.2).

TABLE 4.2

Parameters of the life and fertility table of E. balteatus which were relevant in the present experiment when females were provided with one of the six flower species selected.

x	N(x)						I(x)						m(x)						I(x)m(x)					
	Ph	Bw	Co	Al	Mu	Ma	Ph	Bw	Co	Al	Mu	Ma	Ph	Bw	Co	Al	Mu	Ma	Ph	Bw	Co	Al	Mu	Ma
1	12	12	12	12	10	12	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
2	12	9	11	11	9	6	1.00	0.75	0.92	0.92	0.90	0.50	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
3	12	7	11	10	5	5	1.00	0.58	0.92	0.83	0.50	0.42	18.33	0.86	22.00	11.20	40.00	0.00	0.00	20.24	9.29	20.00	0.00	
4	9	7	11	8	2	4	0.75	0.58	0.92	0.67	0.20	0.33	112.78	19.71	69.45	13.00	21.50	0.00	0.00	63.90	8.71	4.30	0.00	
5	7	7	11	5	2	3	0.58	0.58	0.92	0.42	0.20	0.25	76.86	69.00	76.82	31.80	13.50	2.33	44.58	40.02	70.67	13.36	2.70	0.58
6	6	7	9	4	0	2	0.50	0.58	0.75	0.33	0.00	0.17	62.17	81.00	43.22	18.25	0.00	30.00	31.08	46.98	32.42	6.03	0.00	5.10
7	5	7	7	4	0	1	0.42	0.58	0.58	0.33	0.00	0.08	124.80	51.86	32.86	14.75	0.00	33.00	52.42	30.08	19.06	4.87	0.00	2.64
8	5	6	4	4	0	1	0.42	0.50	0.25	0.33	0.00	0.08	106.80	62.00	13.25	18.50	0.00	8.00	44.86	31.00	3.31	6.11	0.00	0.64
9	4	5	1	3	0	1	0.33	0.42	0.08	0.25	0.00	0.08	96.50	94.20	141	30.67	0.00	13.00	31.85	39.56	11.28	7.67	0.00	1.04
10	3	5	0	2	0	1	0.25	0.42	0.00	0.17	0.00	0.08	29.67	60.60	0.00	0.50	0.00	2.00	7.42	25.45	0.00	0.09	0.00	0.16
11	1	5	0	1	0	0	0.08	0.42	0.00	0.08	0.00	0.00	100.00	51.80	0.00	29.00	0.00	0.00	8.00	21.76	0.00	2.32	0.00	0.00
12	1	4	0	1	0	0	0.08	0.33	0.00	0.08	0.00	0.00	179.00	70.00	0.00	33.00	0.00	0.00	14.32	23.1	0.00	2.64	0.00	0.00
13	1	4	0	1	0	0	0.08	0.33	0.00	0.08	0.00	0.00	105.00	29.75	0.00	2.00	0.00	0.00	8.40	9.82	0.00	0.16	0.00	0.00
14	1	2	0	1	0	0	0.08	0.17	0.00	0.08	0.00	0.00	5.00	33.00	0.00	20.00	0.00	0.00	0.40	5.61	0.00	1.60	0.00	0.00
15	1	1	0	1	0	0	0.08	0.08	0.00	0.08	0.00	0.00	8.00	64.00	0.00	12.00	0.00	0.00	0.64	5.12	0.00	0.96	0.00	0.00
16	1	1	0	0	0	0	0.08	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Ph = phacelia; Bw = buckwheat; Co = coriander; Al = alyssum; Mu = mustard; Ma = marigold.

x is the age class (x = 4 days); n_x is the number of survivors at the beginning of age class x; I_x is the survival rate from age class 0 to the beginning of age x; m_x is the mean numbers of eggs laid per female of age class x.

Correlation between pollen/nectar feeding and performance of female *E. balteatus*

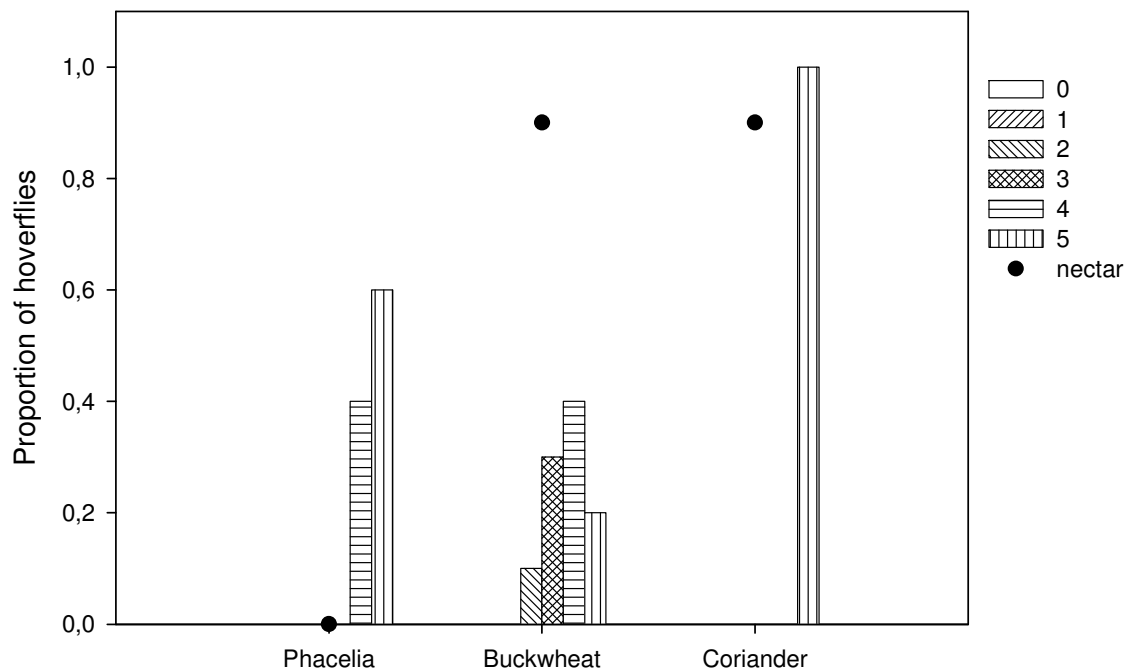


Figure. 4.1. Proportion of female *E. balteatus* having fed on pollen in one of the 6 frequency classes (0 = 0 grain, 1 = 1-10 grains, 2 = 11-100 grains, 3 = 101-500, 4 = 501- 2000, 5 > 2000), and proportion of females having ingested nectar after 24h on one of the three flower species which gave the best reproductive rate.

The different flower species significantly affected the category of pollen in the syrphid guts ($\chi^2 = 47.39$; $P < 0.01$). There was no discernible positive correlation between the quantity of pollen ingested and the resulting female performance. Pollen from the species which gave the best performance, buckwheat and phacelia, were not found in higher quantity in the female guts. A high proportion of female *E. balteatus* (0.9) fed on nectar from buckwheat, coriander and mustard, 0.7 on alyssum and marigold nectars and none on phacelia nectar. Thus Fig. 4.1 displays only results for the syrphids having fed on the floral resources which gave the best performance. Flower species significantly affected the proportion of females which fed on nectar ($\chi^2 = 28.11$; $P < 0.01$).

Discussion

Coriander was the most efficient flower in terms of proportion of females successful in laying eggs. Buckwheat gave the highest mean longevity, which correlated with the longest egg-laying life. However, phacelia increased oviposition rate and lifetime fecundity to the greatest extent, and gave the highest net reproductive rate.

Differences between buckwheat and phacelia in relation to their effectiveness in enhancing *E. balteatus* longevity might be explained by the fact that syrphids did not feed on phacelia nectar: because the labia of hoverflies are not long enough to remove nectar from the phacelia corollae, the plant is likely to be only a pollen resource for syrphids (White *et al.*, 1995). However, syrphids can use sugars from aphid honeydew, which may be less nutritious than is nectar: this was observed for parasitoids (Lee *et al.*, 2004). Honeydew and nectar commonly contain the monosaccharides glucose and fructose, and the disaccharide sucrose (Wäckers, 2001). Honeydew also contains oligosaccharides which are less common to very rare in floral nectar. Several parasitoid species fed on honeydew have a reduced longevity compared with those fed on honey or sucrose solutions and potential reasons for the superiority of nectar include that (1) parasitoids ingest more sugars from floral resources, (2) oligosaccharides in honeydew have a lower nutritional value than do nectar sugars, and/or (3) honeydew may have antagonistic compounds. While oligosaccharides can increase the lifespan of parasitoids, they do so to a lesser extent than do simpler sugars (Wäckers, 2001). These observations reported for parasitoid longevity may explain differences observed on hoverfly longevity between those fed on buckwheat and phacelia. Buckwheat has flowers with shallow corolla tubes, allowing *E. balteatus*, which has a relatively short proboscis compared with other syrphids (Gilbert, 1981) access to nectar and pollen. Females which had fed on phacelia had the highest oviposition rate and lifetime fecundity. Those components of performance must be induced by pollen feeding as phacelia nectar is not accessible to hoverflies.

There was no correlation between pollen and nectar consumption, although this has been observed for brown lacewings (Hemerobiidae) (Robinson, pers. communication).

There was also no correlation between the quantity of pollen ingested and hoverfly performance on each flower species. It seems important to specify that the effect of quantity of pollen consumed on hoverfly performance was not directly measured (i.e., the females tested for performance weren't checked for pollen), but the proportion of females consuming pollen from each flower species was compared with the fitness benefits of each flower species. After 24 h in the presence of each flower species, females had a higher quantity of coriander and mustard pollen grains in their gut; phacelia and alyssum were classified as intermediate, while buckwheat and marigold pollens were ingested to a lesser extent. Nevertheless, phacelia and buckwheat gave the highest results for hoverfly performance, with mustard and marigold the lowest. Colley and Luna (2000) recorded that marigold, compared with coriander, alyssum, phacelia, buckwheat and mustard was relatively undervisited. In the present experiment, given the relatively low number of marigold pollen grains ingested and its low efficacy on hoverfly fitness, the hypothesis that female hoverflies select plant species that offer high quality food resources, which will result in increased reproductive potential, is supported. However, recording the presence of pollen in the diet does not seem to be an adequate method to evaluate hoverfly preference or pollen nutritional value. Results of the present work allow a ranking among these flowers visited, with phacelia, followed by buckwheat and coriander giving the optimal reproductive potential of female *E. balteatus*.

As reported by Cowgill *et al.* (1993a), *E. balteatus* was observed feeding on a range of flower species, but within the range, species were used selectively. Pontin *et al.* (2006) examined the influence of single and multiple species flower treatments on the abundance and foraging behaviour of *M. fasciatum* in agroecosystems; the five flower treatments comprised buckwheat only, phacelia only, a simple mixture of phacelia and buckwheat, a complex mixture of phacelia, buckwheat and a commercial seed blend, or the existing crop as a control. They found that the abundance of hoverflies was similar in all flower treatments and hoverflies probed the flowers of all plant species in single and multiple species treatments, with no apparent difference in acceptance. However, in mixture treatments, the majority of individual hoverflies probed the flowers of only one species, despite the presence of alternative species. This flower constancy, an individual-level response, is common among insect pollinators (Chittka *et al.*, 1999) and Delaplane and Mayer (2000) explained that changes in floral attractiveness by

individual pollinators might occur during a day and over a growing season but not during a single foraging bout.

Hoverflies exhibit a high degree of selectivity, but this 'preference' is clearly influenced by what other flowers are available (Colley & Luna, 2000): thus hoverfly floral preferences fluctuate. This is not surprising as populations of insects which are associated with arable systems will experience both temporal and spatial heterogeneity in flower resource abundance on a range of scales. Rotenberry (1990) studied the degree of temporal resource heterogeneity in a system consisting of a community of insects and the plants they visit for pollen and nectar. He recorded that although insects foraging on the plants throughout the floral season could expect constancy in the relative timing of blooming, they were exposed to considerable temporal heterogeneity in flower resource abundance, both between and within years. He concluded that this heterogeneity would select for an extreme specialist or a broad generalist. This might be expected to affect the behaviour and the composition of the insect community and may be reflected in the observation that species such as *E. balteatus* are generalists.

In the field, hoverflies have been observed feeding on a range of weeds (Holloway, 1976; Gilbert, 1981; Cowgill, 1990; Cowgill *et al.*, 1993a; Hickman *et al.*, 1995; Wratten *et al.*, 1995; Branquart & Hemptinne, 2000b) so one might expect that those visits were correlated with the nutritional value of the weeds, as well as with nectar and pollen accessibility. This observation has implications for the production of guidelines for the management of field margins. Cowgill *et al.* (1993a) identified which of the commonly occurring arable weeds and hedgerow plants were selected by *E. balteatus* in the U.K. and their degree of foraging specialization. Some weed species were used selectively, thus guidelines which encourage the growth of these species should favourably modify the agroecosystem for syrphids. In practice, the management of field margins involves some degree of compromise; the strategies must be economically and agronomically acceptable to farmers. A potential solution may be the use of specially prepared seed mixture in conjunction with appropriate mowing regimes and/or herbicide use which permit the growth of the selected plant species while controlling weeds which are considered unacceptable by farmers (Harwood *et al.*, 1992). The adoption of The Game Conservancy Trust's 'Conservation Headland' technique (Sotherton *et al.*, 1989) which encourages the growth of annual, arable weeds including some of those which are

used selectively by *E. balteatus* can be an alternative. This technique, which involves the selective use of herbicides in the headlands (areas of the field in which farm machinery turns and crop productivity is often lower because of the resulting soil compaction) of cereal fields, is acceptable to farmers and widely established in the U.K. If flowering weeds could increase hoverfly performance to the same extent that phacelia did, it would be ecologically and economically more interesting at the landscape scale to leave some fallow strips treated selectively to encourage the growth of the selected weed species acceptable by farmers and used by syrphids, rather than sow extra floral resource subsidies. However information on the nutritional value of the weeds is non-existent and a similar study comparing phacelia and some of the weeds from which hoverflies were observed feeding would be useful to determine if the weeds can give such reproductive potential. In this case the management of fallow strip should be considered as a method of conservation biological control.

From the insectary plants compared in the present work, phacelia has the best potential to increase hoverfly reproductive success, and so if one floral resource subsidy has to be introduced into agricultural and horticultural systems, the use of phacelia is recommended, at least for *E. balteatus*.

The effect of phacelia and buckwheat flowers on hoverfly abundance in the field

Maintaining a diversity of habitats on farmland increases populations of beneficial insects, and it has been recognised for some time that field margins and their surrogates (e.g., beetle banks; Thomas *et al.*, 1991, 1992) can play an important role in the development of novel manipulation techniques to enhance populations of insect predators and parasitoids (Powell, 1986).

Adult hoverflies feed on nectar and pollen and females require these food sources in order to mature their eggs, which are then laid amongst aphid colonies in the case of aphidophagous species. Access to appropriate non-prey food sources will also increase the ‘fitness’ of the adult flies, allowing them to live longer, fly further and lay more eggs (Scholz & Poehling, 2000). Field margins can supply these food sources in the form of wild flowers and so the presence of flower-rich margins should enhance the impact of hoverflies on aphid populations in nearby crops, by increasing hoverfly abundance and/or increasing their reproductive ‘fitness’.

Evidence from the literature indicates that the provision of added floral resources enhances local hoverfly populations (Molthan & Ruppert, 1988; Sengonça & Frings, 1988; Lövei *et al.*, 1992b; MacLeod 1992; Cowgill *et al.*, 1993b; White *et al.*, 1995; Hickman & Wratten, 1996; Holland & Thomas, 1996; Morris & Li, 2000). Recent work has identified a number of key flower species as important sources of pollen and nectar for the adult flies. Adult hoverflies feed selectively (Haslett, 1989b; Cowgill *et al.*, 1993a), so efficient manipulation of the habitat requires a knowledge of preferences, especially of gravid females, thereby maximising the biocontrol potential by the larvae. Several flowering plant species have been trialed for use in enhancing hoverfly numbers, including phacelia, coriander and buckwheat (Lövei *et al.*, 1992a, 1993; MacLeod, 1992; Hickman *et al.*, 1995; Colley & Luna, 2000; Wratten *et al.*, 2003; Ambrosino *et al.*, 2006). Earlier experiments in this thesis aiming to screen some

potential selective food plants for their effectiveness at enhancing the fitness of adult *E. balteatus* (Chapter 4) showed that buckwheat gave the highest mean longevity and egg-laying life, while phacelia increased oviposition rate and lifetime fecundity to the greatest extent. Those findings highlight the observation that female hoverflies select plant species that currently offer high quality food resources, which should result in increased oviposition rate.

Floral ‘preferences’ reported for hoverfly species from different places such as the United States, Europe or New Zealand are similar. Thus, it was assumed that observations made in the laboratory in France with *E. balteatus* might also be valid for the New Zealand species *M. fasciatum* and *M. novaezelandiae*.

This study investigated the effect of flowering phacelia and buckwheat on hoverflies in a broccoli crop in New Zealand. Hoverfly abundance and sex-ratio were recorded in plots with and without flowers. ‘Preferences’ in flower feeding were assessed by recording numbers of hoverflies caught in traps containing phacelia and/or buckwheat pollen. Also, dispersal of the female hoverflies and the proportion of gravid females captured in each treatment were analysed. Unfortunately, sampling of hoverfly eggs and aphids in the broccoli was not feasible.

Materials and methods

Nine plots (5 m x 5 m) of broccoli (*Brassica oleracea* var. *Italica*) plants were prepared at the Lincoln University Biological Husbandry Unit, in summer from January to March 2004. A 1-m wide strip of a flowering plant species was sown in the last week of January 2004 along two opposite edges of each of the broccoli plots (Fig. 5.1). Three broccoli plots were bordered with *P. tanacetifolia* (cv. Balo) and three with *F. esculentum* (cv. Katowase); the last three plots remained without flowering plants and were the controls. The buckwheat was flowering by 1 March and the phacelia by 11 March. Yellow water traps were used to attract and catch hoverflies. The traps used in this experiment showed high reflectance from 520 nm up to and including 700 nm (White *et al.*, 1995). Traps were placed in the crop when the buckwheat and/or phacelia

plants began flowering and were emptied weekly until 8 April. One trap was placed in the centre of each flower strip, two extended into the broccoli plot, one at 0.5 m from the strip and the second in the middle of the plot. A line of traps extended out of the plot at distances of 0.5 m, 1.5 m, 3.5 m, 7.5 m, 12.5 m and 17.5 m from the strip (Fig. 5.1). These particular distances were chosen, with higher numbers of traps in the vicinity of the flower strips, because hoverfly activity was suspected to be more intense in the vicinity of the floral resources. Flowering weeds on which Syrphidae could forage were cut down in and around the nine plots. To limit interactions between treatments, plots were separated by at least 90 m. Traps consisted of two-litre yellow plastic containers (170 × 170 × 85 mm) which were three-quarters filled with water, 0.5 ml of detergent and 10 % mono-propylene glycol as a preservative. The containers were held in position with an outer container of the same size and colour which was anchored to the ground using a bent metal rod through its centre. Traps were emptied and refilled weekly. The trap contents were strained through a fine mesh sieve and transferred to 70 % ethanol in plastic storage pots. In the laboratory, adults of *M. fasciatus* and *M. novaezelandiae* were extracted individually using forceps and all other insects discarded. Hoverflies were identified to species; they were placed individually on glass slides and sexed using a binocular microscope. Head and thorax together were removed carefully, two drops of 0.0025 % aqueous safranin were added and the abdominal contents dissected out and spread over the slide (Hickman *et al.*, 1995; Wratten *et al.*, 1995; Wratten *et al.*, 2003). One drop of Aquatex®, an aqueous inclusion agent, was added and each slide was then covered with a glass coverslip. The number of eggs in ovaries was recorded.

The first objective was to verify if the total number of *M. fasciatus* was affected by the treatments. A two-way ANOVA was carried out to compare the total number of *M. fasciatus* caught in each plot irrespective to their sex. (Dependent variable: total number of flies; independent variable: treatments and blocks). This analysis was repeated for *M. novaezelandiae*.

The second objective was to check if the sex ratio of the total capture of *M. fasciatus* realized in each plot was affected by the nature of the treatment. A two-way ANOVA was carried out to compare the sex ratio of *M. fasciatus* captured in each treatment. (Dependent variable: total number of flies; independent variable: treatments and

blocks). Prior to the analysis, the proportions were arcsine transformed. The same analysis was done with *M. novaezelandiae*.

The third objective was to assess if the proportion of females having fed in the flower strip and subsequently captured was similar in all the traps. The presence of phacelia and buckwheat pollen was compared with other pollen species found in syrphid guts using a χ^2 test of homogeneity. Then the proportion of female *M. fasciatum* which had fed on the pollen of phacelia and buckwheat captured at each distance was analysed using a χ^2 test of homogeneity. This general table of contingency was subsequently partitioned into contrasts to assess the impact of pollen in general and the impact of phacelia or buckwheat pollen.

Then the focus was put on the proportion gravid females in the traps. The proportion of gravid females of *M. fasciatum* was analysed using a χ^2 test of homogeneity applied to a 3 x 7 Contingency Table.

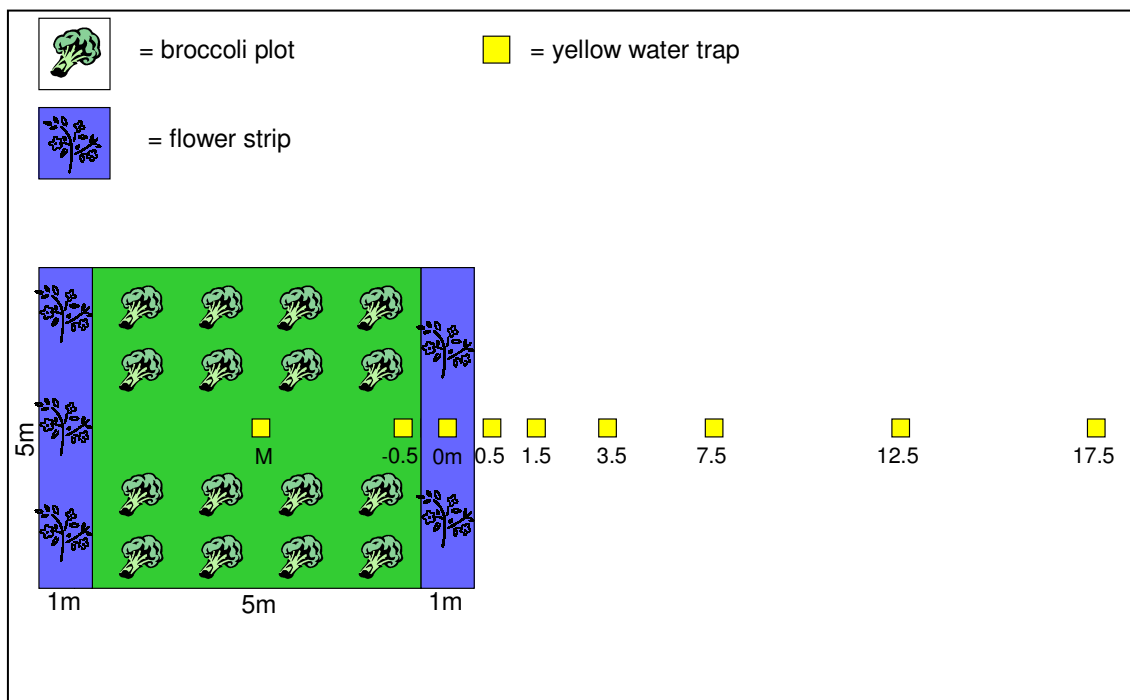


Figure 5.1. Plan of one experimental replicate, showing dimensions and location of the broccoli plot, phacelia/buckwheat strips and the sampling transect which was established across the experiment.

Results

Temporal pattern of hoverfly catches

Between 1 March and 8 April 2005, a total of 771 hoverflies were caught. *M. fasciatum* and *M. novaezelandiae* respectively comprised 567 (74 %) and 204 (26 %) of the hoverflies captured (Table 5.1).

TABLE 5.1

Numbers of male and female M. fasciatum and M. novaezelandiae caught in the phacelia, buckwheat and control plots over the whole trapping period.

	<i>M. fasciatum</i>			<i>M. novaezelandiae</i>		
	♀	♂	Total	♀	♂	Total
Phacelia	114	117	231	29	27	56
Buckwheat	118	107	225	53	32	85
Control	71	40	111	32	31	63
Total	303	264	567	114	90	204

Hoverfly abundance and sex-ratio

When total numbers of *M. fasciatum* caught in phacelia, buckwheat and control plots were compared there were no significant differences between treatments ($F = 2.168$; $df = 2, 4$; $P > 0.05$). Neither there was difference between the blocks ($F = 1.156$; $df = 2, 4$; $P > 0.05$). There was no significant difference between the total numbers of *M. novaezelandiae* captured in phacelia, buckwheat and control plots ($F = 2.45$; $df = 2, 4$; $P > 0.05$). Neither there was difference between the blocks ($F = 1.156$; $df = 2, 4$; $P > 0.05$).

The sex ratio of the individuals *M. fasciatum* captured in each plot was not affected by the treatment ($F = 1.25$; $df = 2, 4$; $P > 0.05$) nor by the blocks ($F = 0.07$; $df = 2, 4$; $P > 0.05$). The same observations were done for *M. novaezelandiae*, the sex ratio was not affected by the treatment ($F = 2.25$ $df = 2, 4$; $P > 0.05$) nor by the blocks ($F = 2.25$ $df = 2, 4$; $P > 0.05$).

Female hoverflies and pollen feeding

If attention was given to the total number of hoverflies caught in each treatment (with and without the presence of the specified pollen), similar numbers of female *M. fasciatum* were captured in the plots bordered with phacelia or buckwheat (Table 5.1), and a higher number of female *M. novaezelandiae* were captured in the buckwheat plots. Thus, the proportion of female *M. fasciatum* and *M. novaezelandiae* which contained the pollen of phacelia or buckwheat in their guts was analysed for each treatment. A significantly higher proportion (0.69) of female *M. fasciatum* found in the phacelia plots had fed on this pollen than on any other ($\chi^2 = 8.82$; $df = 1$; $P < 0.01$; Fig. 5.2), while for the females found in the buckwheat plots, a higher proportion (0.61) had not fed on buckwheat pollen, but differences were not significant ($\chi^2 = 2.9$; $df = 1$; $P > 0.05$) (Fig. 5.2). The proportion of female *M. novaezelandiae* which had consumed the pollen of phacelia and buckwheat was 0.21 and 0.13, respectively. Thus, the majority of female *M. novaezelandiae* did not feed on phacelia or buckwheat pollen (Fig. 5.2) (phacelia: $\chi^2 = 5.45$; $df = 1$; $P < 0.05$; buckwheat: $\chi^2 = 16.59$; $df = 1$; $P < 0.01$).

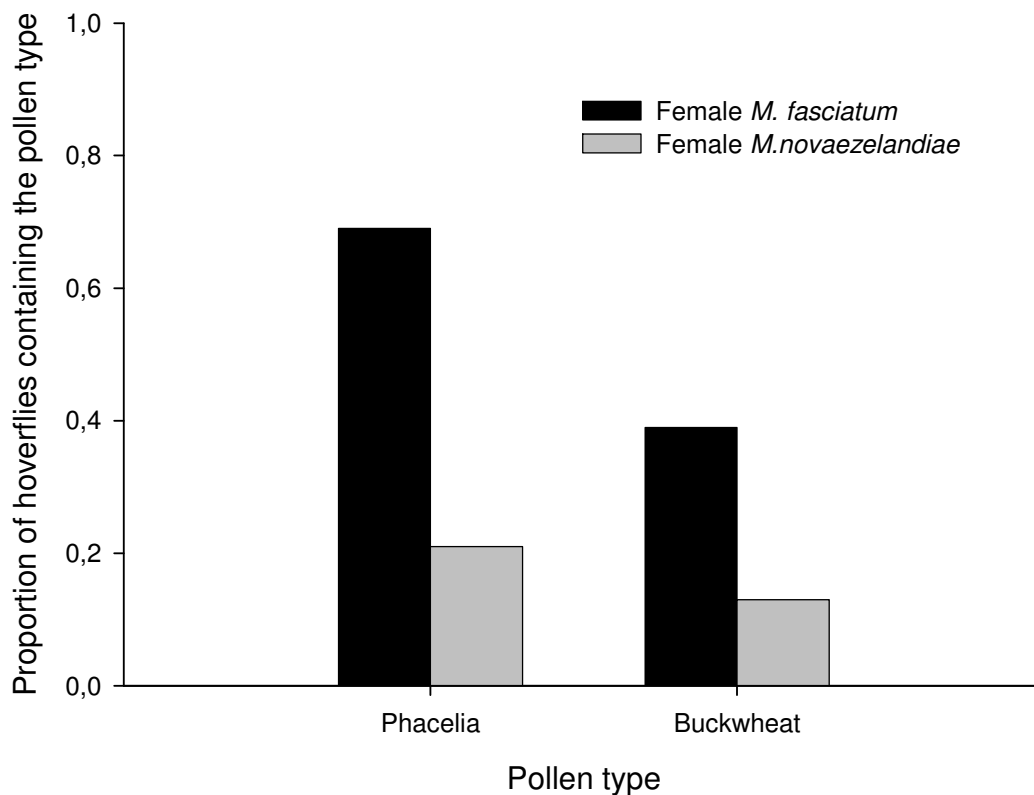


Figure 5.2. Proportion of female *M. fasciatum* and *M. novaezelandiae* which had fed on phacelia and buckwheat pollens and which were caught in water traps.

Distance had no effect on the proportion of female *M. fasciatum* which had consumed or not the pollens of phacelia and buckwheat ($\chi^2 = 23.1$; $df = 24$; $P > 0.05$; Table 5.2). When attention was given only to the females which had fed on the pollen of phacelia and buckwheat, whatever the pollen consumed, distance had no effect on the proportion of female *M. fasciatum* caught at each distance. Moreover, that hoverflies consumed or not pollen, distance did not affect the proportion of female *M. fasciatum* caught at each distance.

TABLE 5.2

Results of the Table of contingency analyzing the proportion of female M. fasciatum which had fed on the pollen of phacelia and buckwheat

	Results of the analysis
Total χ^2	23.1 ; 24 d.f. ; $P > 0.05$
Presence phacelia vs presence buckwheat	6.28 ; 8 d.f. ; $P > 0.05$
Presence pollen vs absence pollen	7.86 ; 8 d.f. ; $P > 0.05$

Individuals with phacelia pollen were found up to 17.5 m, and except at positions + 7.5 m and + 12.5 m, the majority of females caught did consume phacelia pollen (see Fig. 5.3). A different pattern was observed with females which had fed on buckwheat pollen. Most of the females caught did not consume this pollen (Fig. 5.4). Because of the low number of *M. novaezelandiae* captured, it was impossible to determine the effect of distance on the proportion of females which had consumed phacelia and buckwheat pollen. As shown in Figures 5.5 & 5.6, no pattern of *M. novaezelandiae* dispersal was evident

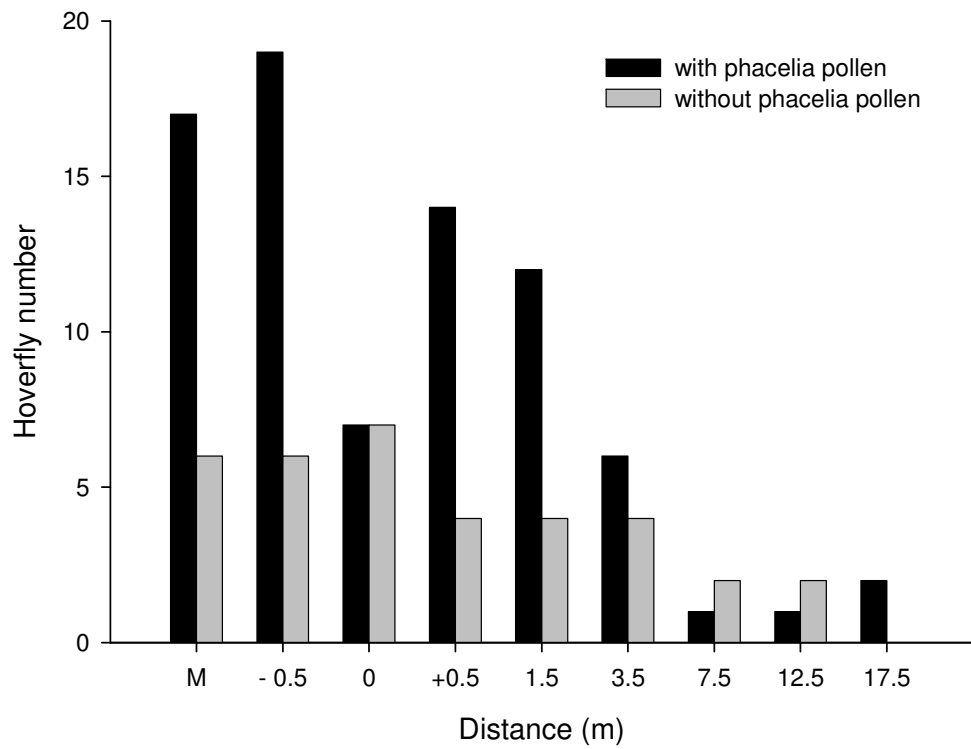


Figure 5.3. Numbers of female *M. fasciatum* with and without phacelia pollen over distance.

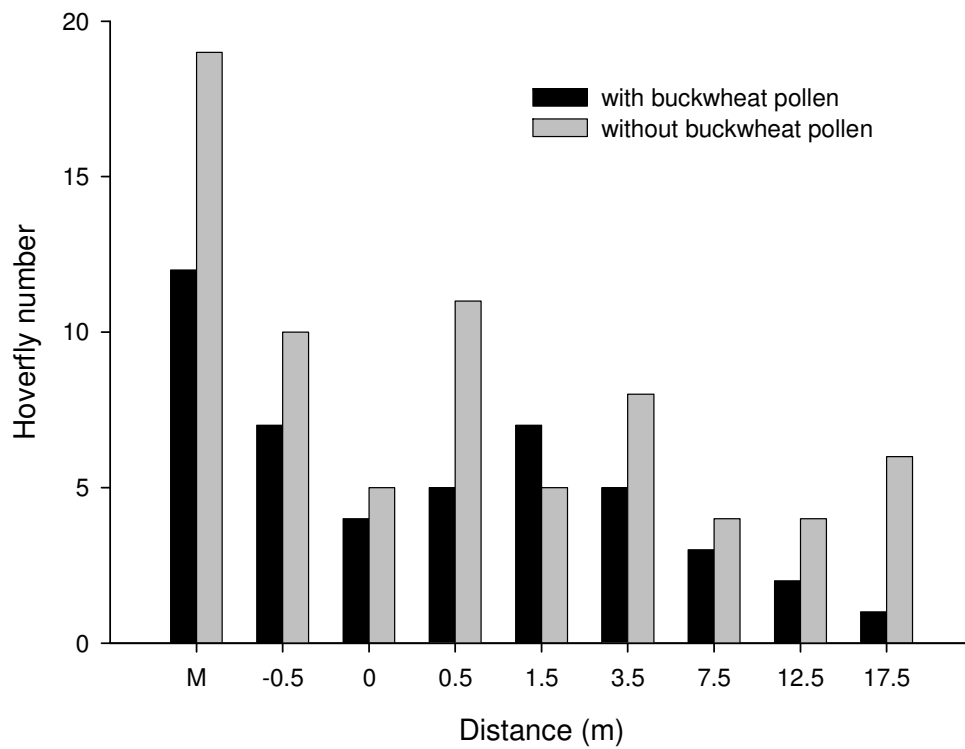


Figure 5.4. Numbers of female *M. fasciatum* with and without buckwheat pollen over distance.

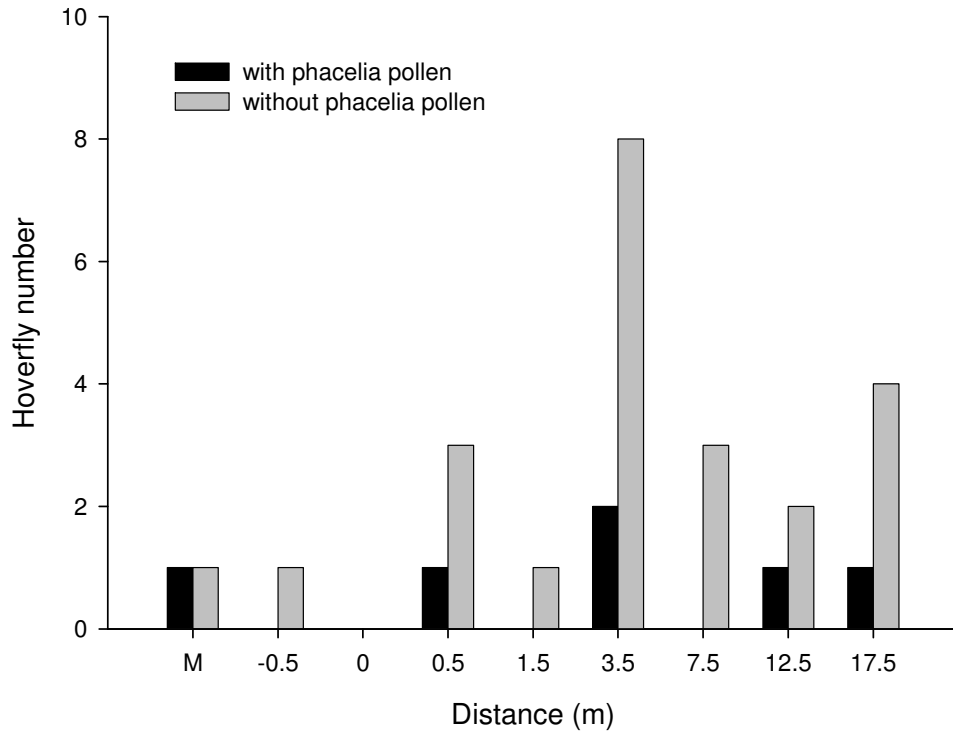


Figure 5.5. Numbers of female *M. novaezelandiae* with and without phacelia pollen over distance.

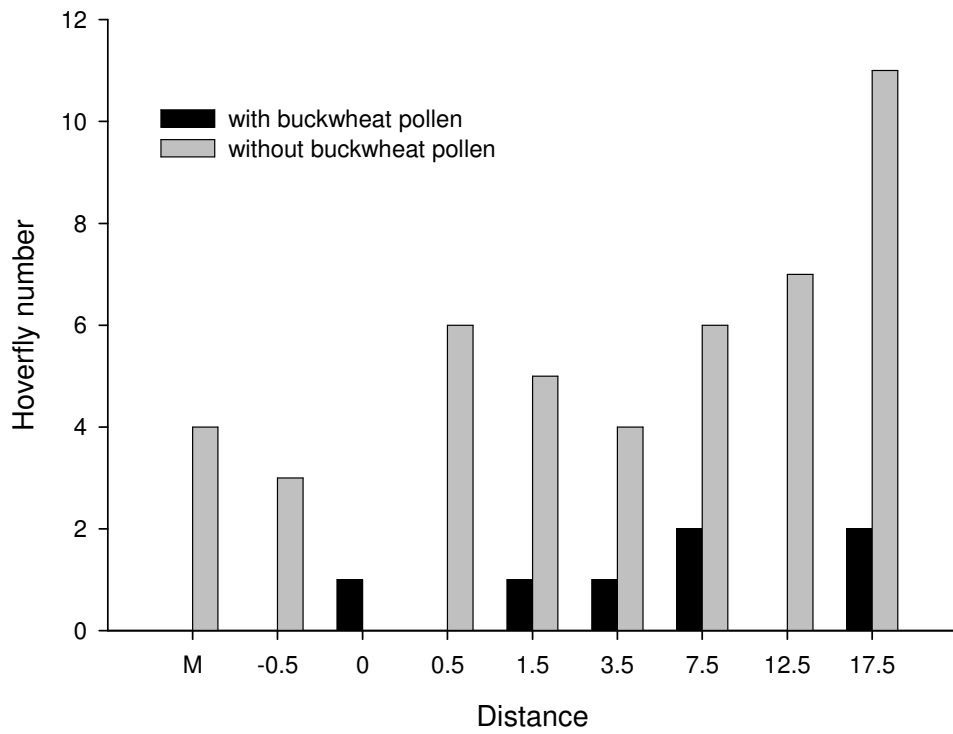


Figure 5.6. Numbers of female *M. novaezelandiae* with and without buckwheat pollen over distance.

TABLE 5.3

Numbers of gravid and non gravid hoverflies caught at different distances from the floral strip in the three treatments.

Distance from strip	<i>M. fasciatum</i>						<i>M. novaezelandiae</i>					
	Phacelia		Buckwheat		Control		Phacelia		Buckwheat		Control	
	Gravid	NG	Gravid	NG	Gravid	NG	Gravid	NG	Gravid	NG	Gravid	NG
Middle	19	5	20	4	14	2	1	1	2	1	0	1
-0.5	18	5	8	3	×	×	2	0	2	1	×	×
0	11	2	4	1	×	×	0	0	1	0	×	×
+0.5	14	3	8	4	10	1	1	1	2	0	1	3
+1.5	10	2	10	1	7	0	0	1	0	1	0	1
+3.5	6	3	10	3	5	0	4	5	1	1	2	1
+7.5	1	0	5	4	11	1	3	0	2	4	0	3
+12.5	2	0	4	2	8	2	2	1	1	1	3	2
+17.5	1	1	7	1	7	1	3	2	2	1	5	6
Total	82	21	76	23	62	7	16	11	13	10	11	17

× : no traps at these positions (because there was no flowering strip in this treatment). NG = non gravid.

Effects of phacelia and buckwheat on the proportion of gravid and non-gravid females of *M. fasciatum* and *M. novaezelandiae* in the field.

Because of the low number of gravid female *M. novaezelandiae* caught in the experiment, statistical analysis was possible only for *M. fasciatum*. In each treatment, gravid females of *M. fasciatum* were significantly more abundant than non-gravid (phacelia: $\chi^2 = 19.8$; df = 1; P < 0.01; buckwheat: $\chi^2 = 15.28$; df = 1; P < 0.01; control: $\chi^2 = 26.06$; df = 1; P < 0.01; Table 5.3).

TABLE 5.4

*Results of the Table of contingency analyzing the proportion of gravid female *M. fasciatum* which had fed on the pollen of phacelia and buckwheat*

	Results of the analysis
Total χ^2	22.76 ; 12 d.f. ; P < 0.05
Flowers (Pha + Bw) vs Control	14.62 ; 6 d.f. ; P < 0.05
Phacelia vs buckwheat	9.55 ; 6 d.f. ; P > 0.05

The proportion of gravid female *M. fasciatum* captured in the traps was significantly different between the treatments ($\chi^2 = 22.76$; df = 12; P < 0.05; Table 5.4).

When comparisons were made between the gravid female of *M. fasciatum* captured in the flower treatments against the control, the distance affected the numbers caught ($\chi^2 = 14.62$; $df = 6$; $P < 0.05$). However, when comparisons were made between gravid female of *M. fasciatum* captured in phacelia and buckwheat treatments only, distance had no effect on the proportion of female *M. fasciatum* caught ($\chi^2 = 9.55$; $df = 6$; $P > 0.05$)

High numbers of gravid female *M. fasciatum* were observed in the vicinity of the flower strips; more gravid females *M. fasciatum* were always found in the middle of the broccoli plot, while lower numbers were trapped in the strip itself (0 m).

Gravid females of both species were detectable up to 17.5 m, reflecting their ability to disperse from floral resources.

Discussion

Hoverfly abundance and sex-ratio

Only a limited positive effect of flowers on hoverfly abundance was found, mostly involving *M. fasciatum*. More individuals of *M. fasciatum* were found in phacelia and buckwheat compared with control plots, although differences were not significant between the three treatments. This observation is consistent with previous studies which have shown that provision of added floral resources enhances local hoverfly populations (Molthan & Ruppert, 1988; Sengonça & Frings, 1988; Lövei *et al.*, 1992b; MacLeod 1992; White *et al.*, 1995; Hickman & Wratten, 1996; Morris & Li, 2000).

Sex-ratio was analysed because if many more males than females are found in the flower vicinity, this would be potentially a negative result in conservation biological control. For both species, similar numbers of males and females were captured and there was no effect of flowers on the sex-ratio.

Female hoverflies and pollen feeding

Although similar numbers of female *M. fasciatum* were found around the plots bordered with phacelia or buckwheat, and a higher number of female *M. novaezelandiae* were

trapped around the buckwheat plots, females of both species consumed more phacelia pollen than buckwheat. Moreover, hoverfly gut analysis revealed that 69 % of the female *M. fasciatum* captured in the phacelia plots had fed on this pollen, while only 39 % of the female captured in the buckwheat plots had fed on buckwheat pollen. These proportions were 21 % and 13 % for the female *M. novaezelandiae* caught in the phacelia and buckwheat plots, respectively. These observations suggest that many of the flies trapped did not come from the flower strip but were attracted to the crop by the yellow traps. Other possibility was that hoverflies were attracted to the floral patch, but that they were moving towards it, rather than away from it.

High numbers of female *M. fasciatum* which had fed on phacelia pollen were found at each distance, up to 17.5 m, and distance did not affect the proportion of female *M. fasciatum* having consumed phacelia pollen. On the other hand, most of the females caught did not consume buckwheat pollen and distance did not affect these proportions. The fact that distance did not affect numbers of female hoverflies caught is not surprising as the study was constrained by the size of the experimental area. However results on *M. novaezelandiae* are difficult to analyse given the low number of individuals caught. The work of Wratten *et al.* (1995) on hoverfly foraging indicated that *M. fasciatum* is less mobile compared with *M. novaezelandiae*.

Effects of phacelia and buckwheat on the proportion of gravid and non-gravid females of *M. fasciatum* and *M. novaezelandiae* in the field.

The proportion of gravid females was not significantly different between the treatments. This result is conflicting as one could have expected more gravid females in the plots bordered with floral resources, meaning they visited the pollen and nectar sources. Alternatively, more gravid females in the control plots might be expected, which would have suggested that the main stimulus for this category of flies was prey, not flowers. The fact that gravid females were present in plot with and without flowers suggests that flowers enhanced the activity of gravid female hoverflies but also that gravid females foraged for oviposition sites. This is supported by two observations: first, a higher proportion of gravid female *M. fasciatum* was always found in the middle of the broccoli plot, compared with other trapping positions, suggesting again that oviposition site was the key stimulus. Second, high numbers of gravid females were also found close to the flowers (-0.5 m; +0.5 m), meaning that floral resources probably increased

visit frequencies of gravid females, or led them to aggregate. This observation supports the work of Hickman *et al.* (1995), who showed that pollen is of great dietary importance for gravid *M. fasciatum* females. The low numbers of hoverflies trapped in the strip itself (0 m), phacelia as well as buckwheat, might be explained by the fact that traps were hidden by the floral resources.

In each treatment, gravid female *M. fasciatum* were significantly more abundant than non-gravid. This is a positive result in terms of conservation biological control.

The floral resources added, especially phacelia, enhanced the activity of female *M. fasciatum* and *M. novaezelandiae*. The high numbers of females found around the phacelia strip suggest that those hoverflies forage for phacelia pollen: because the labia of hoverflies are not long enough to remove nectar from the phacelia corollae, the plant is likely to be only a pollen resource for syrphids (White *et al.*, 1995). Or may be the flies were attracted to the flowers by their colour and scent, expecting nectar, but then they found they were unable to acquire it. However, honeydew from aphids within crops might provide an alternative source of energy for the flies, so the absence of nectar does not negate the use of phacelia in biological control (Harwood *et al.*, 1994). Results with gravid females suggest that they visited phacelia and buckwheat flowers, but also that they required oviposition sites.

In the flower ranking experiment (Chapter 4), phacelia increased oviposition rate and lifetime fecundity of female *E. balteatus* to a large extent, compared with buckwheat. The present results, indicating that females of *M. fasciatum* and *M. novaezelandiae* consumed more phacelia pollen than buckwheat in the field, support the choice of phacelia as an ideal floral resource subsidy in crops for enhanced biological control by hoverflies.

The effect of floral resource subsidies on the field distribution of adult *M. fasciatum* and *M. novaezelandiae*

Beneficial insectary planting is a form of conservation biological control that involves introducing flowering plants into agricultural and horticultural systems to increase the nectar and pollen resources required by most natural enemies of insect pests. Surveys of naturally occurring weed and wild plant communities in agroecosystems indicate that the abundance of flowers in non-crop habitats is positively correlated with the abundance of 24 pollen and nectar-feeding natural enemy species in and around fields (Cowgill, 1989; Cowgill *et al.*, 1993b) and orchards (Leius, 1967). Attracting and retaining natural enemies into crop are key goals of conservation biological control. However, for it to be successful, and for practitioners to understand the reasons for success (or failure) it is necessary in many cases to have a sound knowledge of natural enemy locomotory behaviour and spatial distribution, as well as of abundance (Jervis *et al.*, 2004). Once attracted by the resource, the natural enemies are expected to leave it and to attack the pest in surrounding crops. The distances they are usually able to travel will determine the location of these resources and the surface devoted to these resources.

Little is known about the movement of hoverflies after they have visited a flower resource and in particular whether they are likely to move within or into a field and oviposit there. In most of the previous works, experiments focused on global hoverfly populations, while information is required on females and more specifically on gravid females. Their numerical response might be a crucial factor in determining the outcome of biocontrol. Wratten *et al.* (2003), working on the role of field boundaries on hoverfly dispersal, showed that 45 % of the *M. fasciatum* captured 1 m from a phacelia strip had eaten phacelia pollen, against 1.1 % at 180 m. Lövei *et al.* (1993) found similar overall proportions of flies with 'marker' pollen, with phacelia or coriander pollen being found in hoverflies trapped in a wheat crop up to 75 m from the flower source. This indicates that some flies travel great distances from the flower strips and a similar trend was observed in the UK with *E. balteatus* and *M. corollae*. The work of Hickman and

Wratten (1996) in winter wheat crop confirmed this: more adult syrphids were caught in plots bordered with phacelia than in control plots, indicating that the influence of phacelia extended to at least 180 m from the border strip.

Although many hoverflies feed on floral resource subsidies and have the ability to disperse within the crop, it does not follow that hoverflies, and more specifically gravid females, are able to travel between the floral subsidies and the crop in sufficient numbers and with a homogeneous distribution, to improve pest suppression. Several authors have suggested that hoverflies may accumulate in florally rich field margins during their flower feeding phase, and subsequently fail to disperse into the crop effectively during their egg laying period. In most of the previous works, hoverflies remained on the floral resource and very few numbers were found more than 10 m from the floral strip (Lövei *et al.*, 1993; Harwood *et al.*, 1992; Harwood *et al.*, 1994; White *et al.*, 1995; Wratten *et al.*, 2003). Moreover, apart from the work of Lövei *et al.* (1993), the studies did not focus on gravid female dispersal. Once again further whole crop studies are required to confirm this.

The present study was set up on a commercial field scale, provided by a grower which had to produce biological carrot seeds. This gave us the possibility to observe hoverfly dispersal, and more particularly of gravid females, in another type of culture, the carrots. Moreover the focus was made on the proportion of flies having eaten the floral resource pollen, with the aim to generalise hoverfly efficacy.

Several studies in New Zealand have demonstrated that *P. tanacetifolia* is attractive to the native hoverfly species (Lövei *et al.*, 1992a, 1993; Hickman *et al.*, 1995; Wratten *et al.*, 2003). Pollen of phacelia is easily distinguished from all pollens found in arable and horticultural areas in most parts of the world, thus providing a good biological marker. The main advantage of pollen is that it is self-marking, moreover, it is tough, durable and it is not likely to affect predator behaviour or survival (Silberbauer *et al.*, 2004). This plant therefore fits the criteria for an effective pollen marker, which are that it must be a plant species taxonomically distant from other species and from plant species in the vicinity. Moreover, several studies have shown that gut examination for pollen grain types and quantity is feasible for hoverflies that have been caught in yellow water-traps (Wratten *et al.*, 1995; Hickman & Wratten, 1996; Irvin *et al.*, 1999; Hickman *et al.*,

2001; Wratten *et al.*, 2003). Thus, flies were marked via their ingestion of the pollen of *Phacelia tanacetifolia*.

This research was done to determine the proportion of male and female *M. fasciatum* and *M. novaezelandiae* which fed on selective floral resources, and to investigate their dispersal from floral resources into a carrot field, particularly of gravid females which are of interest for CBC. The following questions are addressed: once attracted by floral resource subsidies, do hoverflies remain in the surrounding area, laying eggs among aphid colonies, in numbers high enough to sustain aphid predation pressure? Or do they feed but then disperse to find other suitable floral resources?

Materials and methods

A carrot (*Daucus carota*) field 450 × 270 m was provided by a farmer near Ashburton, Canterbury, New Zealand. Four 1-m wide strips of tansy leaf, *P. tanacetifolia* (cv. Balo) were planted 100 m apart, this design giving 3 large blocks (Fig.6.1). Traps used to assess hoverfly activity were two-litre plastic containers (170 × 170 × 85 mm). One was used as a trap base and anchored to the ground with a bent metal rod through its centre. A second was inserted into the base and was three-quarters filled with water, 0.5 ml of domestic detergent and 0.5 ml mono-propylene glycol as a preservative. Traps were aligned at 0, 1, 5, 10, 20 and 50 m between each of three pairs of phacelia strips on 3 November 2004. Two lines of water traps (at least 70 m apart) were placed in each block: one line with both containers yellow and one line with outer container green and inner container yellow. Yellow-green traps were first chosen for this kind of experiment because they would be more appropriate to reflect the local abundance of hoverflies (Laubertie *et al.*, 2006). However in case not enough hoverflies were caught for statistical analysis, yellow traps, which are highly attractive (Finch, 1992), were added. The traps were weekly emptied in storage pots between 11 November 2004 and 25 January 2005. Then they were refilled with the mixture of water, detergent and mono-propylene glycol as described above.

In the laboratory, adults *M. novaezelandiae* and *M. fasciatum* were extracted individually using forceps and all the other insects discarded. Hoverflies were identified

to species; they were placed individually on glass slides and sexed using a binocular microscope. Head and thorax were removed carefully, and the abdominal contents dissected out and spread over the slide (Hickman *et al.*, 1995; Wratten *et al.*, 1995; Wratten *et al.*, 2003). Two drops of 0.0025 % aqueous safranin were added, followed by one drop of Aquatex® and each slide was then covered with a glass coverslip. The number of eggs in ovaries was counted. Gut fullness was estimated on a semi-quantitative scale by classifying pollen number as accurately as possible into one of the 5 frequency classes (1 = <10 grains, 2 = 11-100 grains, 3 = 101-500, 4 = 501- 2000, 5 > 2000). Phacelia pollen was recorded based on its characteristic pollen-coat pattern (Wratten *et al.*, 2003). The morphological features of the exine make it easy to spot phacelia pollen grains in syrphid gut. As a consequence, it was possible to record the presence or absence of this pollen on the microscopic preparation.

Because of dilution of marked insects by non-marked ones with distance from the flowers, absolute numbers of marked flies were not the metric used. Rather, the rate of decline with distance and maximum distance travelled were considered more important. In the present experiment there was no control but three experimental blocks, in which distance was the variable recorded. Insect numbers in yellow-yellow traps and yellow-green traps, so called YY and YG, respectively, and sex-ratio were analysed using one-way analyses of variance. Where numbers of insects were sufficient, count data were analysed using a Generalised Linear Model with Poisson error distribution and a Log link. The proportion of insects with phacelia over the experimental distances was compared using GLM with binomial error and a Log link. χ^2 was used to analyse the proportion of insects that contained phacelia pollen, the proportion of hoverflies having fed on more than 2000 pollen grains (Class 5) and the proportion of gravid females in the population.

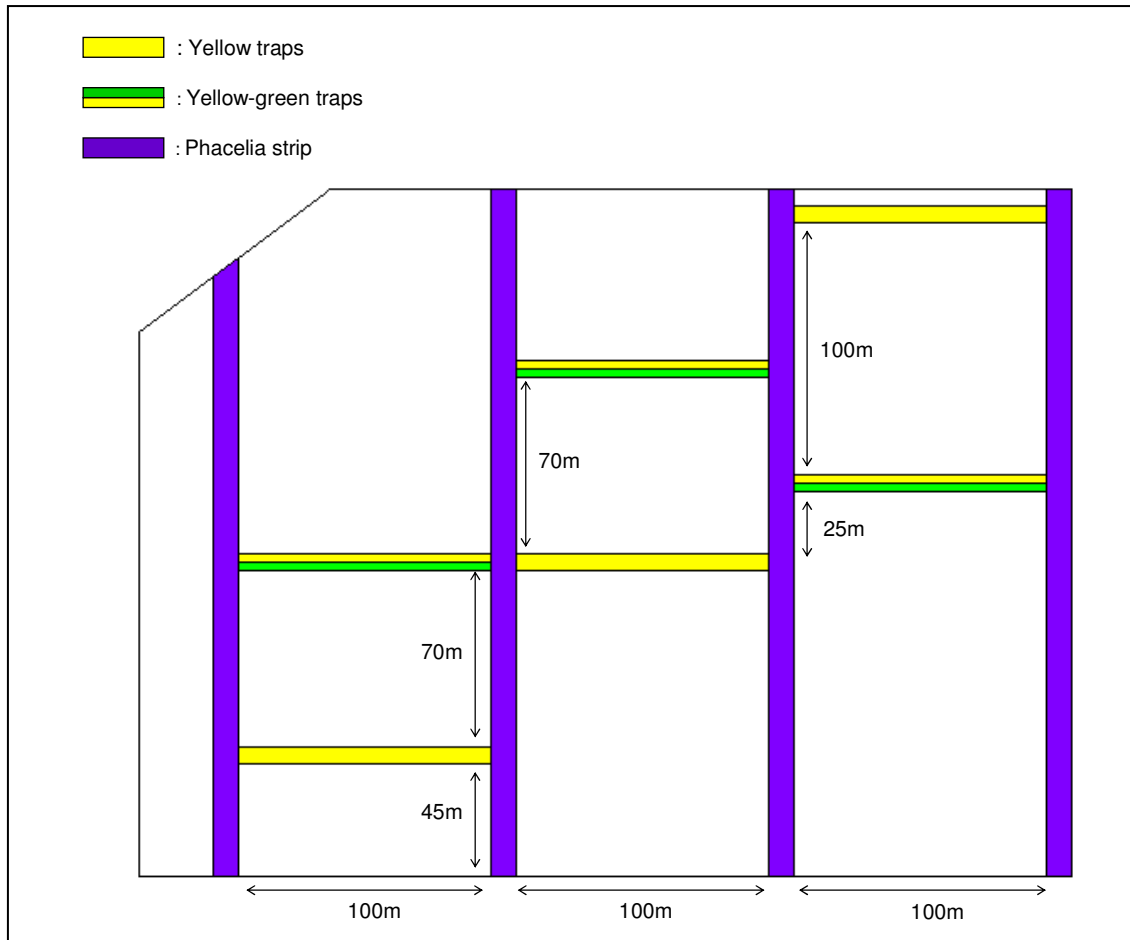


Figure 6.1. Plan of the carrot experimental site, showing arrangement of yellow and yellow-green trapping network between the phacelia strips which was established across the experiment.

Results

Temporal pattern of hoverfly catches

Between 11 November 2004 and 25 January 2005, a total of 927 hoverflies were caught. *M. fasciatum* and *M. novaezelandiae* respectively comprised 352 (38 %) and 575 (62 %) of the hoverflies captured (Table 6.1).

Difference between yellow traps and yellow-green traps

The number of hoverflies caught in completely yellow and yellow-green traps were not significantly different (*M. novaezelandiae*: $F = 0.591$; $df = 1, 64$; $P = 0.445$; *M. fasciatum*: $F = 0.685$; $df = 1, 64$; $P = 0.411$) (Table 6.1). Therefore, the numbers of syrphids caught in both types of traps were combined.

TABLE 6.1

The number of gravid and non-gravid hoverflies of M. fasciatum (Mf) and M. novaezelandiae (Mn) trapped in yellow and yellow-green traps between 10 November 2004 and 25 January 2005.

	Mf ♀		Mf ♂	Mf total	Mn ♀		Mn ♂	Mn total
YY	61		96	157	178		126	304
	gravid	not gravid			gravid	not gravid		
	37	11			102	40		
YG	31		164	195	170		101	271
	gravid	not gravid			gravid	not gravid		
	12	10			102	40		
Total	92		260	352	348		227	575

Note: differences between total numbers of female hoverflies and total numbers of gravid + non-gravid females are due to the fact that many insects were damaged and often only head and thorax were left, so only their sex could be determined.

Sex ratio

Significantly more male than female *M. fasciatum* were caught throughout the experiment ($F = 13.422$; $df = 1, 64$; $P = 0.001$), while for *M. novaezelandiae* significantly more females were captured ($F = 10.528$; $df = 1, 64$; $P = 0.002$) (Table 6.1).

TABLE 6.2

Proportion of phacelia pollen and egg load in adults of M. fasciatum and M. novaezelandiae in relation to the capture site within the field.

	Distance from phacelia strip					
	0	1 m	5 m	10 m	20 m	50 m
<i>M. fasciatum</i>						
males with pollen	0.39	0.48	0.29	0.17	0.24	0.13
females with pollen	0.67	0.44	0.55	0.15	0.31	0.2
females with eggs	1	0.56	0.45	0.84	0.81	0.4
gravid females with pollen	0.67	0.31	0.27	0.15	0.25	0
<i>M. novaezelandiae</i>						
males with pollen	0.49	0.55	0.35	0.29	0.31	0.6
females with pollen	0.44	0.26	0.25	0.29	0.13	0.17
females with eggs	0.77	0.57	0.75	0.75	0.77	0.69
gravid females with pollen	0.31	0.17	0.19	0.2	0.12	0.14

Hoverfly distribution

GLM analysis of log-transformed total trap catches vs log distance from a phacelia strip revealed a significantly greater proportion of male *M. fasciatum* with phacelia in the 1 m traps than in the 10 m and 50 m traps ($P < 0.05$). The proportion of male *M. novaezelandiae* with this pollen tended to be lower at the 50 m traps but differences were not significant. However, there was a highly significant effect of distance in the model-fitting process ($P < 0.01$). The proportion of female *M. novaezelandiae* with phacelia was greater at 0 m than at 20 m or 50 m ($P < 0.05$), while for female *M. fasciatum*, the number of flies captured was insufficient for statistical analysis. However the greatest proportion of female of *M. fasciatum* with phacelia occurred at 0 m, 1 m and 5 m (Table 6.2). Males and females of both species with phacelia pollen in their gut were trapped up to 50 m from the flower strip.

TABLE 6.3

Numbers (percentage) of gravid and non gravid hoverflies of each species, with and without P. tanacetifolia pollen in their gut, trapped between 10 November 2004 and 25 January 2005.

Pollen	Mf ♀ gravid	Mf ♀ not gravid	Mf ♂	Mf total	Mn ♀ gravid	Mn ♀ not gravid	Mn ♂	Mn total
Present	20 (41)	7 (33)	80 (36)	107 (36)	54 (26)	21 (26)	63 (37)	138 (30)
Absent	29 (59)	14 (67)	144 (64)	187 (64)	150 (74)	59 (74)	108 (63)	317 (70)
				294				455

Pollen gut content of hoverflies

Thirty-six percent of *M. fasciatum* captured had eaten phacelia pollen while the figure for *M. novaezelandiae* was 30 %. For both species, there was no significant heterogeneity between the three reproductive/sex classes in the percentage of individuals whose guts contained pollen (see Table 6.3 for proportions). The gut of 41 % of gravid females of *M. fasciatum* contained pollen; this figure was 33 % for non-gravid females and 36 % for males ($\chi^2 = 0.54$, $P > 0.05$). For *M. novaezelandiae*, 37% of the males had consumed phacelia pollen, compared to 26 % of gravid and no-gravid females.

Most of the hoverflies captured had not eaten on phacelia pollen; however if the percentage of females having fed on pollen in relation to their position of capture within the field is analysed, 44 % of female *M. novaezelandiae* trapped at 0 m from the flower strip contained this pollen, with 67 % for females *M. fasciatum* (Table 6.2).

Differences in pollen feeding

Only 41 % of male *M. fasciatum* compared with 78 % of gravid and 62 % of non-gravid females were in the highest class of pollen frequency in the gut content ($\chi^2 = 24.12$; $P < 0.01$) (Fig. 6.2). These percentages were 56 %, 79 % and 65 %, respectively, for *M. novaezelandiae* ($\chi^2 = 21.53$; $P < 0.01$) (Fig. 6.3).

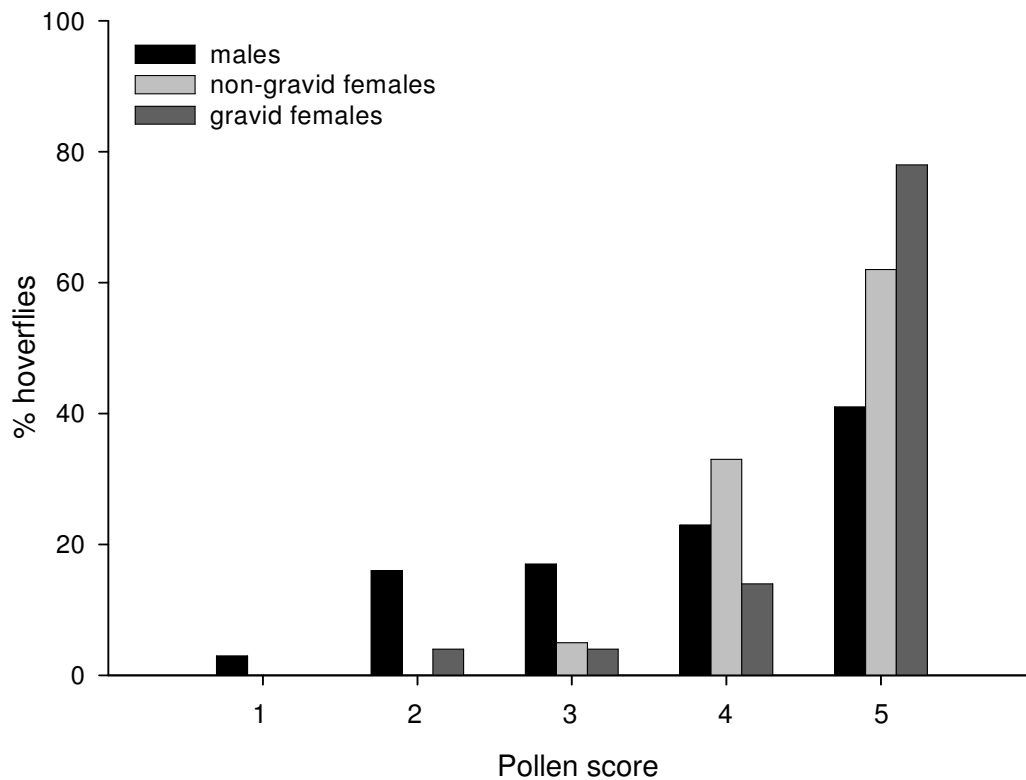


Figure 6.2. Frequency distribution of the number of pollen grains in male, non-gravid and gravid female *M. fasciatum*.

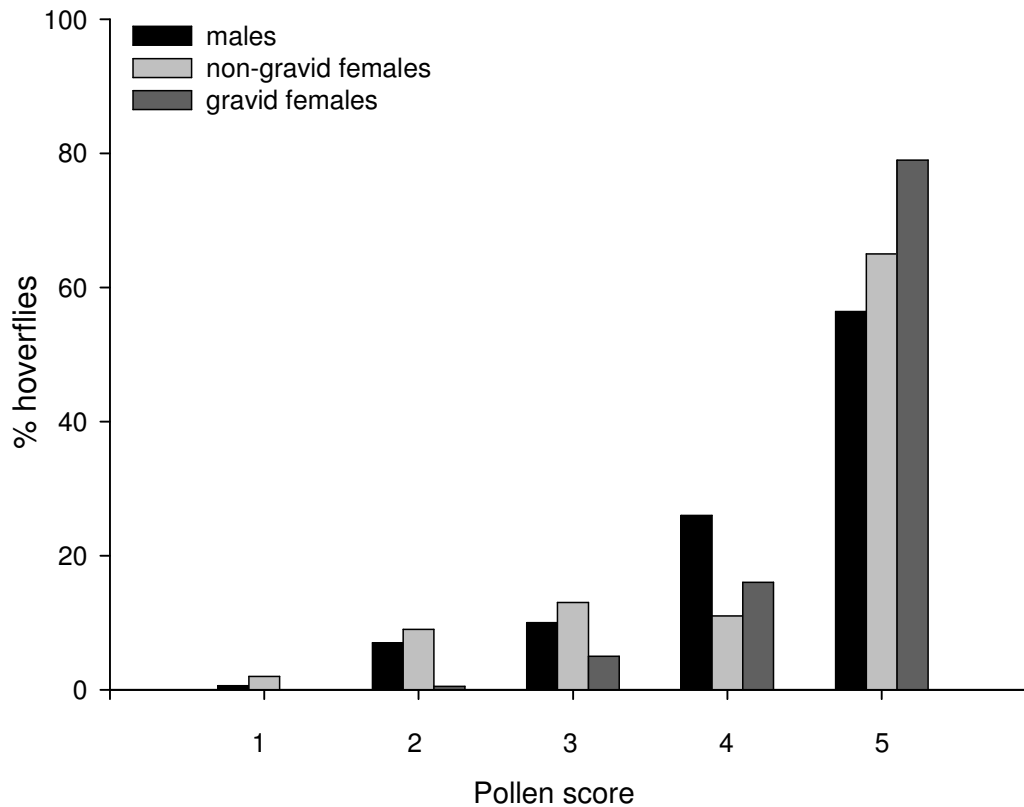


Figure 6.3. Frequency distribution of the number of pollen grains in male, non-gravid and gravid female *M. novaezelandiae*.

Proportion of gravid females in the population

The proportion of gravid female *M. fasciatum* and *M. novaezelandiae* was respectively 0.7 and 0.72. Over the entire trapping period, a significantly higher proportion of females was gravid (*M. fasciatum*: $\chi^2 = 6.18$; $P < 0.05$, *M. novaezelandiae*: $\chi^2 = 29.02$; $P < 0.01$).

Proportion of gravid females collected at each sampling date

All the female *M. fasciatum* trapped were gravid from 10 to 24 November; then this proportion fell, but from 21 December, it increased again to reach 0.68 and 0.71 over the last capture dates (Fig. 6.4). For female *M. novaezelandiae* this trend is reversed with a maximum of gravid females between 17 November and 28 December, with peaking proportions at 0.77 and 0.86.

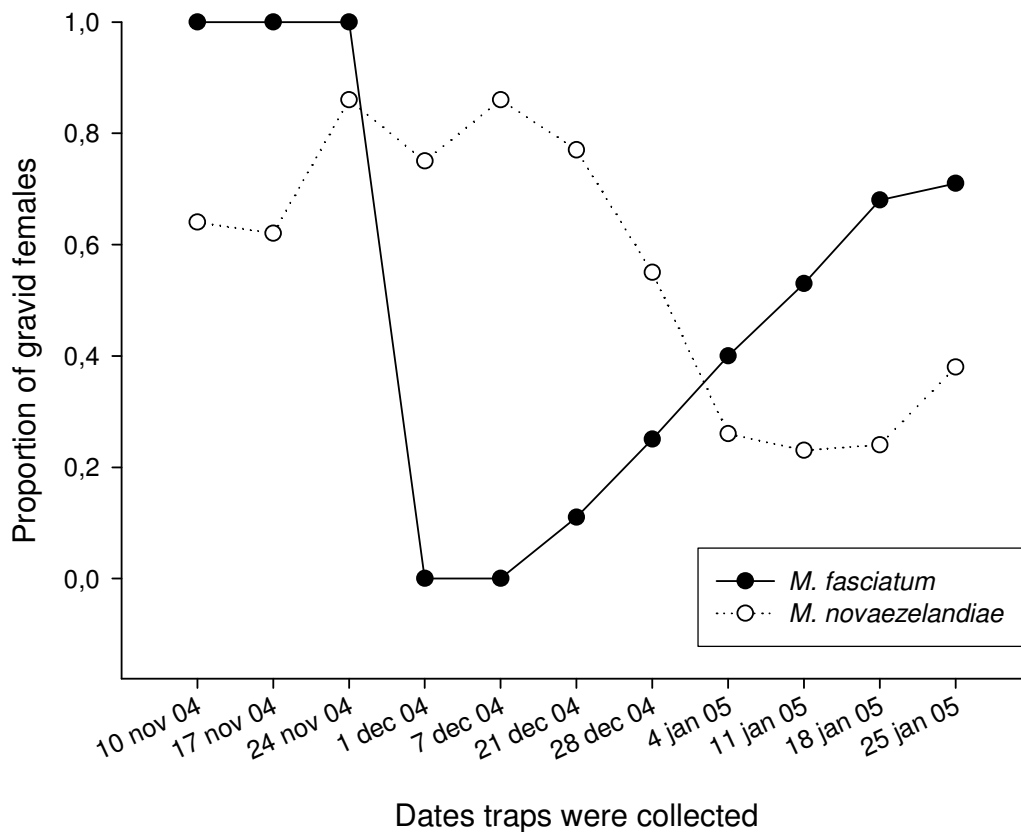


Figure 6.4. Proportion of gravid females *M. fasciatum* and *M. novaezelandiae* captured weekly over the experiment.

Proportion of gravid females having fed on phacelia pollen over the distance

Figure 6.5 was realised using data when gravid females of each species were the most abundant (from 10 to 24 November 2004 and from 28 December to 25 January 2005 for *M. fasciatum* and between 17 November and 28 December 2004 for *M. novaezelandiae*). The number of flies caught during this sampling period being higher, this gave a clearer trend. It appears that at the edge of the phacelia strip, the proportion of gravid female *M. fasciatum* and *M. novaezelandiae* captured which had eaten phacelia pollen was 0.71 (n = 5) and 0.29 (n = 9), respectively (Fig. 6.5). The proportion decreased over distance to 0.17 at 20 m and 0 at 50 m from the flower strip for *M. fasciatum* and to 0.12 at 20 m and 0.12 at 50 m for *M. novaezelandiae*. Given the low number of female *M. fasciatum* captured all over the experiment, the data for *M. novaezelandiae* may give a more appropriate idea of the proportions of hoverflies feeding on and flying from the flower strips.

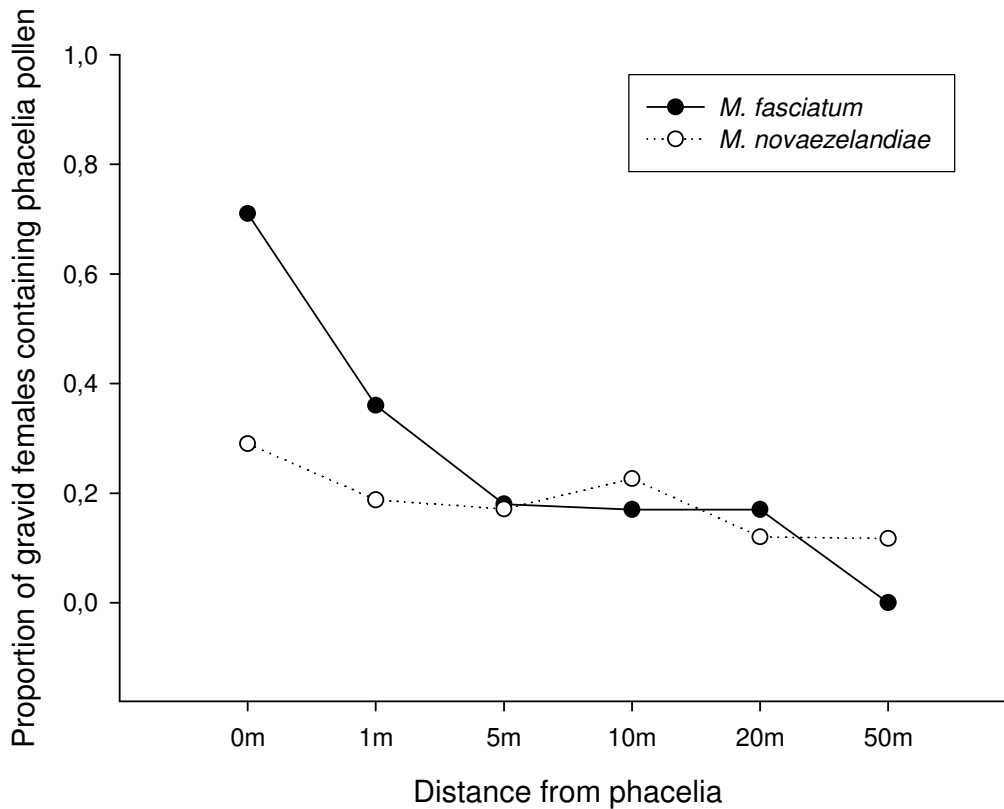


Figure 6.5. The relationship between the proportion of gravid female *M. fasciatum* and *M. novaezelandiae* that contained phacelia pollen in relation to distance from a phacelia strip.

Discussion

Differences between YY/YG traps

Though more hoverflies were expected in YY traps than in YG traps, this was not the case, as numbers caught in completely yellow and yellow-green traps were not significantly different. A possible explanation is that adult hoverflies could not detect the difference between both colours of traps. Containers were anchored to the ground and from the beginning of November the carrot plants were as high as the traps, hiding the external walls.

Sex ratio

For conservation biological control by aphidophagous hoverflies to be useful, it is essential that an enhanced number of females travel into the crop to lay their eggs near

aphid colonies. Therefore, the need to estimate proportions of males and females in the population at all distances was identified. Results are quite disturbing as observations made for the two species are different: significantly more female than male *M. novaezelandiae* were caught throughout the experiment, while for *M. fasciatum* significantly more males were captured.

Powell *et al.* (2004) in a similar experiment observed that many more males than females were captured in the yellow water traps and compared the sex-ratio of the hoverflies in the traps in the crop with that from suction samples. They found a large difference, as the water traps gave a ratio male/female of around 1.9:1 while suction sampling gave a ratio male: female of around 0.5:1. This difference can possibly be accounted for by the hypothesis that the yellow water trap represents a food signal to the hoverflies (Hickman *et al.*, 2001) and that the majority of females that fly into the crop from margins are responding to oviposition signals rather than food signals. However, males flying away from the flowering strips are probably more interested in females than in food. Powell *et al.* (2004) supplemented their study by laboratory works, which showed that gravid females made significantly fewer visits to traps (food signals) even though they had previously been starved. Two-day old, non-gravid, females, however, were as strongly attracted to the traps as were males. They concluded, therefore, that all the males in the crop and only the newly emerged females were responding to food signals, resulting in a greater number of males being captured in the water traps and that the more mature gravid females were more likely to be responding to oviposition signals and searching amongst the canopy for aphid colonies.

Results of the present work cannot confirm this hypothesis. Firstly, because the analysis of hoverfly pollen content showed that gravid females of *M. fasciatum* and *M. novaezelandiae* did not make less use of pollen than did non-gravid females and males. Secondly, because differences observed between both species can hardly be explained; maybe it was too early in the season to catch enough female *M. fasciatum*. However, hoverflies hibernate as gravid females to be ready to lay eggs as soon as they resume activity.

Dispersal

The results from regression analysis indicated that hoverfly numbers were greatest close to the phacelia strips. This is not surprising as hoverflies are well known to be highly attracted by floral resources, especially *P. tanacetifolia*. (Frank, 1999; Lövei *et al.*, 1992a, 1993; MacLeod, 1992; Hickman *et al.*, 1995; Hickman & Wratten, 1996; Wratten *et al.*, 2003). White *et al.* (1995) also captured highest numbers of hoverflies in the traps closest to the phacelia strips and concluded that the flies do not disperse very far from such a pollen resource. However hoverflies are highly mobile and their experiment was rather small-scale, with traps placed up to 12.5 m from the flower strip. In the present experiment hoverflies dispersed at least 50 m from the flowers. Lövei *et al.* (1993) trapped flies with phacelia or coriander pollen up to 75 m from the flower source. In another related study, phacelia pollen was found in the guts of *M. fasciatum* up to 180 m from the source; a similar trend was observed with *E. balteatus* and *M. corollae* in the UK where pollen was recorded up to 200 m (Wratten *et al.*, 2003). Another example involving *E. balteatus* recorded the presence of phacelia pollen up to 250 m from the plants (Harwood *et al.*, 1994).

It supports that hoverflies have the ability to disperse widely from flower sources, but maybe they disperse too widely to remain in the crop itself. The relatively low proportion of hoverflies with the pollen marker captured at 50 m from the flower strip established that hoverflies have dispersed far from the crop. However, due to the fact that four phacelia strips were planted in this experiment, exact distances travelled by hoverflies cannot be precise. Experiments in which hoverflies are marked automatically (e.g., via rubidium chloride (Graham *et al.*, 1978; Freeman-Long *et al.*, 1998; Lavadero *et al.*, 2004b) would be appropriate for investigating the distances over which these insects can disperse into the crop.

Pollen content of hoverflies

Contrary to the data of White *et al.* (1995) who suggested that gravid females make less use of pollen than do non-gravid females and males, gravid female *M. fasciatum* and *M. novaezelandiae* had more pollen in their guts than did non-gravid females and males. Hickman *et al.* (1995) also showed that pollen intake did not seem to be lower in gravid females. Hoverflies are synovigenic and require a protein source to mature their eggs whereas males need nectar to provide energy for mate seeking. European hoverfly

species have similar traits (Haslett 1989a); in *Rhingia campestris* (Meigen), pollen ingestion was greatest at the time of yolk deposition in the eggs, with peaks of nectar feeding at the beginning and end of oogenesis. Presumably the first peak was associated with finding a mate, while the second would be related to oviposition behaviour. It appears that pollen is of high dietary importance for gravid females.

However most of the hoverflies captured had not fed on phacelia pollen: 26 % of the gravid females of *M. fasciatum* and 41 % of those of *M. novaezelandiae* contained the pollen in their guts. A possible explanation is that hoverflies feed on a large variety of pollen species (Cowgill *et al.*, 1993a; Hickman *et al.*, 1995; Colley & Luna, 2000), reducing the relative attraction of phacelia flowers. If the proportion of gravid females having fed on phacelia pollen in relation to their position capture in the field is compared, 71 % of gravid females of *M. fasciatum* were trapped at 0 m from the flower strip compared with 29 % of those of *M. novaezelandiae*. However, because of the low number of female *M. fasciatum* captured over the experiment, those data have to be dealt with care and do not allow any generalization.

A significantly higher proportion of female hoverflies trapped were gravid (0.7 vs 0.3), but only gravid female *M. novaezelandiae* which had fed on phacelia pollen were found up to 50 m. As we discussed for the sex ratio, the use of yellow water-traps may not be appropriate to assess the numbers of gravid females captured into the crop, as yellow traps are perceived as food sources (Hickman *et al.*, 2001; Laubertie *et al.*, 2006). If gravid females have been shown to feed on pollen in the flower strips, the majority of gravid females that fly into the crop from strips are responding to oviposition signals rather than food signals. If the sequence from day to day of hoverfly adult life in terms of flower use and prey seeking was known, that would help. Such information is missing but experiments using laboratory video, gut pollen and sugar analysis can contribute to have a better understanding of hoverfly behaviour.

Those results support the idea that hoverflies, and, more specifically, gravid females which are of interest for biological control consume phacelia pollen. However, even if large quantities of pollen were found in hoverfly guts, the majority of insects did not feed on phacelia pollen. Numbers of hoverflies of each species were relatively low. Although syrphids came in this crop and feed on phacelia pollen, apparently not enough remained

in the surrounding area. Moreover, very few numbers of syrphids were captured at 50 m from phacelia, compared to numbers at the border of the floral strip. One reason would be that yellow traps were not efficient to catch populations of hoverflies flying at this distance. Another possibility is that hoverflies have dispersed far from the crop itself. This would support the hypothesis that syrphids fly to the floral resource to feed on it, but then disperse to find other suitable floral resources. These observations were confirmed by the fact on 13 January 2005, the insecticide Neem had to be sprayed over the crop, as hoverflies failed to reduce aphid populations.

Chapter 7

Hoverfly efficacy in the field and laboratory

There exists a hierarchy of outcomes which researchers hope to achieve when conducting CBC research (Gurr *et al.*, 2003). These steps may be used to determine whether successful conservation biological control has been achieved. They are (1) the aggregation of natural enemies at or near the flowers; (2) an enhancement of natural enemies' 'fitness' (longevity, fecundity and searching efficiency); (3) an increase in parasitism or predation rate in the pest population; (4) a decrease in pest population density; (5) the pest populations are brought below the relevant economic threshold (so avoiding the need to apply curative insecticide). Considerable research effort has focused on syrphid responses to flower strips (Harwood *et al.*, 1992; Hickman & Wratten, 1996; MacLeod, 1999) which are known to increase hoverfly numbers, at least locally. However, it does not follow that more eggs are laid in a field near the floral strips because syrphids are highly mobile and are able to cover great distances. Chandler (1968a) found no difference in oviposition in small replicated plots of Brussels sprouts between those with buckets of flowers added and those without. It is also possible that, after floral resource feeding, they forage for hosts in more distant host patches, or that floral resource subsidies have not been sown in quantities big enough to increase hoverfly visit frequencies and make the flies remaining in the local area. For example, Sutherland *et al.* (2001a) found significantly more hoverflies in field boundaries than in flower patches in the field itself and concluded that if there is a reluctance for *E. balteatus* to move into fields, then this species could be an unsuitable candidate for biological control *via* augmentation. However, they did not assess hoverfly larval or aphid densities in and around the patches.

In an earlier study on dispersal and distribution of *M. fasciatum* and *M. novaezelandiae* in carrot fields (Chapter 6), very few syrphids were found at 50 m from phacelia, compared with numbers near the floral strip. Moreover, the majority of insects did not feed on phacelia pollen, although large amounts of pollen were found in their guts. Hoverflies visited phacelia flowers, but apparently very few remained in the crop.

The failures observed may have several causes. They can be related to the female optimal foraging theory (Kan & Sasakawa, 1986; Kan, 1988a, 1988b, 1989; Hemptinne *et al.*, 1993), in which they avoid large and/or old aphid colonies which may be already exploited. In the same way, females of some species of aphidophagous ladybirds and chrysopids refrain from ovipositing in patches where conspecific larvae are present; they respond to a specific oviposition deterring pheromone present in the tracks of larvae. This is adaptative as larval cannibalism is a major threat to egg survival (Hemptinne *et al.*, 1992, 2001; Ruzicka, 1996, 1997 & 1998; Doumbia *et al.*, 1998). The failure could also be related to the phacelia strip size, which could have been too small compared with the crop area to exert a sufficient effect.

It was decided to start this study by setting up a field experiment where the proportion of extra-floral resources would be immoderately large compared with the crop area. The hypothesis was that a larger proportion of extra-floral resources should keep hoverflies there, they would lay more eggs and as a result reduce aphid populations.

Many studies have demonstrated the potential of hoverfly larvae as efficient biological control agents of pest aphids. There is evidence of hoverfly effectiveness in slowing aphid multiplication (Chambers & Sunderland, 1982; Chambers *et al.*, 1986); they can prevent population increases (Chambers & Adams, 1986) or actually cause a decrease in aphid populations (Sengonça & Frings, 1988). However the reasons for success or relative failure were never analysed in detail. Few studies have focused on the predator: prey ratio required for control of aphid populations by syrphids. Wnuk (1977) measured the effect of predators on the aphid population in small systems in the laboratory and found that colonies of *Aphis pomi* (DeGeer) were totally destroyed within a few days by *E. balteatus* at predator: prey ratios from 1:50 to 1:200. Chambers (1986) set up preliminary experiments in Perspex cages using aphids and syrphids on single cucumber plants to establish whether *M. corollae* had the capacity to reduce *Aphis gossypii* (Glover). He found that larvae 1, 2 and 3 days old prevented aphid increase unless there were more than 15, 26 or 41 aphids per larva, respectively. Tenhumberg and Poehling (1991) released second-instar larvae of *E. balteatus* in field cages; they found that even at a predator: prey ratio of 1:245, hoverfly larvae greatly reduced aphid density (by 92 %). These experiments highlight the potential of syrphid larvae as efficient aphid predators, but also the significant differences observed between the predator: prey ratios

required to reduce aphid populations, most likely connected to the experimental conditions (laboratory or field work, temperature, time of the year...).

Results of the field experiment set up in this study will provide information on hoverfly fecundity, larval efficacy, ratios of hoverflies: aphids and the development of aphid colonies in the field when optimal food sources are provided to the female syrphids and other natural enemies.

The second part of this study focused on hoverfly larval behaviour. A preliminary experiment was carried out in a greenhouse, with the aim to make a transition between the field and the laboratory experiments. Again the hypothesis that a larger proportion of extra-floral resources should attract more hoverflies, which would lay more eggs, was used to start this series of experiments. Instead of working with several floral resource areas, several densities of first-instar syrphid larvae were compared for their effectiveness in reducing aphid populations. The objective was not to determine one more predator-prey ratio that would be effective to greatly reduce or destroy aphid populations, but to study syrphid larval efficacy in a simplified system, to establish whether larvae of *E. balteatus* could stop population increase of *A. pisum*.

Although hoverfly larvae have the potential to slow aphid population growth, in most of the cases observed, in field but also laboratory experiments, their activity was not sufficient to drastically reduce aphid populations. One reason that could explain this lack of efficiency is the presence of interspecific and conspecific larvae. Cannibalism occurs in a wide variety of predators when prey becomes scarce; in circumstances of starvation, the eating of conspecifics has a survival value (Fox, 1975; Duelli, 1981; Hemptinne *et al.*, 2001). Information about hoverflies and cannibalism is limited. It has been shown that in the laboratory all three instars of *E. balteatus* ate eggs and in the absence of aphids they also consumed younger larvae (Branquart *et al.*, 1997). Another hypothesis was that mutual interference could occur between larvae; as predators aggregate in patches of high prey density, it is increasingly likely that they will encounter each other while searching for prey, which in turn may lead to an increased tendency toward dispersal (Hassel, 1978). When larvae are at high densities, and/or when aphids are scarce, they could avoid each other and try to escape. The common

effect of such mutual interference is to reduce the available searching time in direct proportion to the frequency of encounters. Hassel and Varley (1969; see also Hassel, 1978) have shown how various cases of mutual interference can all be reduced to a common form by calculating the searching efficiency of the consumer and plotting this against consumer density on logarithmic scales (Begon *et al.*, 1996). We would thus expect to find that the searching efficiency per predator over the experimental period declines as predator density increases. This is often the case, at least from laboratory experiments.

The third and last part of this work was carried out in the laboratory, on bean plants and in Petri dishes. Aims were to locate hoverfly larvae among aphid colonies at certain time intervals and to observe hoverfly larvae almost continuously to study their behaviour and answer the following questions: Is there some cannibalism? Do larvae avoid each other and try to escape (mutual interference)? There does not appear to be a study of this type where authors try to understand why syrphid larvae hardly achieved a total control of aphid populations.

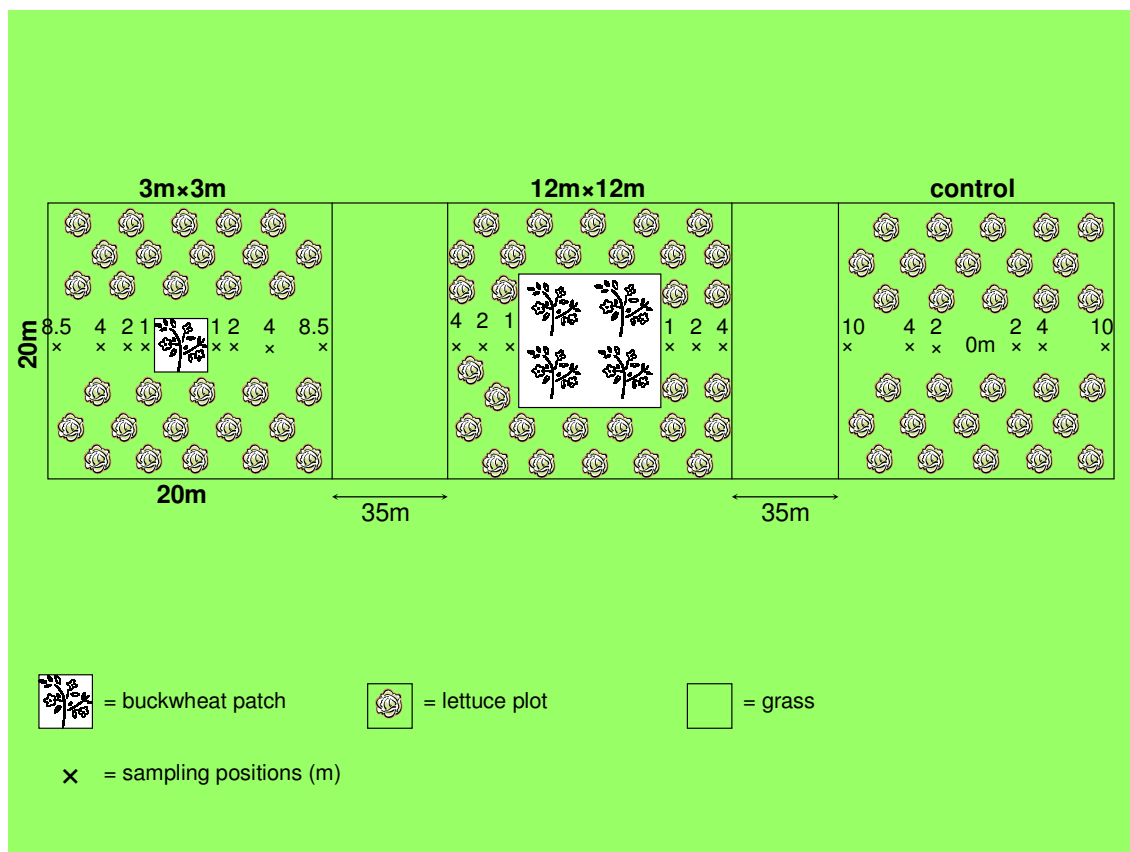


Figure 7.1. Plan of one experimental block, showing dimensions and location of lettuces and buckwheat plots and the sampling positions.

Materials and Methods

1. The role of floral resource area in hoverfly efficacy

Study site

On 15 December 2005 six cultivated areas each of 20 m × 60 m were prepared on experimental land at the Lincoln University Horticultural Research Area. Each area was divided into 3 plots 20 m × 20 m. An area of buckwheat was drilled in the centre of each plot on 28 December 2005. Lettuces (cv. Target) plants were transplanted on 19 and 20 January 2006, with a within-row spacing of 45 cm and 90 cm between rows. There were 3 treatments: a small buckwheat area 3 m × 3 m; a large buckwheat area 12 m × 12 m; a plot without buckwheat for control. The 3 treatments were replicated 6 times each. It is well known that hoverflies are highly mobile and the plots are not very far apart (land-availability restrictions) so flowers were sown in the centre of each plot to reduce interactions between plots. Plots were kept free of other weeds by hoeing.

Sampling of aphids and their natural enemies on lettuce

Lettuces were sampled weekly along a transect between 15 February and 22 March 2006. In the 12 m × 12 m plots, they were sampled at 1 m, 2 m, and 4 m from the edge of the buckwheat area; in the 3 m × 3 m plots, at 1 m, 2 m, 4 m and 8.5 m from the edge of the buckwheat area; and at 2 m, 4 m and 10 m from the centre of the plot in the control. To do this, one lettuce was cut at its base for each sampling position, then each leaf was carefully removed and visually monitored for numbers of hoverfly eggs, hoverfly larvae, aphids, parasitized aphids (mummies) and other natural enemy eggs and larvae such as those of lacewings, ladybirds.

Preliminary one-way analysis of variance was performed to determine if the number of aphids, hoverfly eggs and larvae recorded in each plot varied between traps. The numbers of aphids, eggs or larvae caught in traps in each replicate were not significantly different so the data were pooled and the mean values calculated. The numbers of aphids, hoverfly eggs and larvae sampled in the 12 m × 12 m, 3 m × 3 m and control plots (with transect data pooled first, as described above) were compared using a generalized linear model routine and the significances of the main effects and

interactions determined. All statistical analysis was performed using Systat 9® (Zar, 1996).

2. *Episyrphus balteatus* larval behaviour

2.1. Preliminary experiment: Is hoverfly fecundity high enough to reduce aphid populations?

Experiments were set up in a greenhouse from 10 October until 16 December 2005. A black sheet covered by Perspex was extended on the floor of a single mesh cage 110 × 400 × 100 cm. This allowed tracking of larvae between plants. Plots of broad bean plants were prepared in the greenhouse. To facilitate the experiment, they were sown in 13-cm diameter pots, with two seeds per pot. This allowed continuity in the experiment by the immediate replacement of the plants. Each experimental unit was constructed by placing 20 pots touching together to facilitate insect circulation. They were placed on the sheet. Glue was spread on the sheet all around the experimental unit to prevent aphids and larvae escape. Each experiment was made by a replicate and its control, which gave a total of 80 (seeds) plants per cage. Aphid populations were followed from the time the first-instar larvae were introduced until a few days after pupation, in order to determine whether larvae of *E. balteatus* could stop population increase of *Acyrtosiphon pisum* (Harris). Experiments were not replicated. The focus was made on the overall tendency observed throughout this work.

1. Bean plants inside the cage were infested at 10 aphids *A. pisum* of mixed instars per shoot. First-instar larvae of *E. balteatus* were hand-placed randomly at a density of 0.4 per plant; this corresponds to the natural abundance of syrphid larvae in cereal field (Adams 1984; Tenhumberg 1995) and gave a ratio of predator to prey 1:25. Every two days, aphid density was recorded. At the end of the experiment, plants were dissected to find either alive or dead larvae or pupae. At the same time, a control consisting of bean plants infested at 10 aphids per shoot was set up on the opposite side of the cage. It was ensured that no cross contamination could be possible between treatments as only wingless aphids were present at the time of the experiment, so their only possibility was to walk. Numbers of aphids were recorded every two days until the colony decline; this allowed the examination of aphid development in the absence of predators.

2. From the result of the previous experiment, the number of hoverfly larvae having to be added in the system to reduce aphid populations was estimated. A new experimental unit was added to the cage. It was infested with 10 aphids per plant. *Episyrphus balteatus* larvae were placed at a density of 1.2 per plant, giving a ratio 1:8.3. At the same time, a control consisting of bean plants infested at 10 aphids per shoot was set up in the cage. Aphid density in the treatment and in the control plot was recorded each 2 days until the larvae pupate.

3. Aphid density was increased to 20 aphids per plant. *Episyrphus balteatus* larvae were placed at a density of 1.2 per plant. This gave an initial ratio 1:16.6. A control consisting of bean plants infested at 20 aphids per shoot was set up in the cage. Aphid density was recorded each 2 days until the larvae pupate. The aim of this treatment was to determine if differences in the initial number of aphids inoculated had a strong impact on larval efficacy.

Trends over time are shown in graphs of mean aphid density.

2.2. Hoverfly larvae movements on broad bean plants

The following works were carried out in the laboratory. Aims were to locate hoverfly larvae among aphid colonies depending on time and observe hoverfly larvae almost continuously to study their behaviour. To achieve this, second-instar larvae were used, as first-instar larvae were difficult to observe because of their small size. Moreover, each experimental unit was reduced to five pots (compared with the 20 pots of the previous experiment).

Hoverfly larvae were obtained from a stock culture. Larvae were fed every other day an excess of mixed instars of *A. pisum* until they reached the second instar. Broad bean plants were prepared in a greenhouse, 2 plants were grown in each 13-cm diameter pot and then infested at the third leaf stage at 10 *A. pisum* of mixed instars per shoot. Aphids were left for 24 h, time for the colony to set up. Bean plants were introduced in mesh cages (40 × 75 × 50 cm) on a plinth in a tray then glue was spread on the internal

walls of the tray to prevent larvae escape. Each experimental unit was reduced to 5 pots. *Episyrphus balteatus* second-instar larvae were hand-placed randomly at densities 1.2 per plant, giving the ratio 1:8.3. Experiment was carried on at a constant temperature of $21 \pm 2^\circ\text{C}$ under a long photoperiod (16 h light; 8 h dark).

As larvae are mainly nocturnal, hoverflies and aphids were followed during the dark period using infrared light. On the first day of the experiment, hoverfly larvae were localized and their behavior recorded at 9.00 am, 9.30 am, and then every 2 hours until 3.30 pm. The following days, larvae were localized at 4.00 pm until they reached the pupal stage, i.e. for four days. Aphid density was recorded every day.

The proportion of larvae moving against time was analysed using χ^2 analysis.

2.3. Effects of aphid and conspecific larvae density on the behaviour of hoverfly larvae in Petri dishes

All replicates were performed in Petri dishes 15-cm diameter in which a target was represented. Three circles were drawn: the smallest was 3-cm diameter, then 6 cm and 10 cm. This allowed us to describe where and when encounters did occur and how the larvae did react. A third-instar larva of *E. balteatus* starved for 2 hours was placed in the middle of the Petri dish and was offered 2 or 8 second-instar larvae of *E. balteatus* and 0 or 40 mixed instar aphids. This gave us four treatments.

There were 20 replicates of each treatment. A piece of broad bean plant was added to each dish as a source of food for the aphids and humidity for the larvae. Every 30 min hoverfly larvae were localized and the following behaviors recorded: (a) did they avoid each other? (b) did they have contact? And if they did: (c) did they attack each other? (d) was there some cannibalism? (e) did they try to escape ? If the fugitive larva covered more than 6 cm it was considered as leaving the system and was discarded. Mortality and pupation were recorded.

The proportion of third-instar larvae of *E. balteatus* remaining on the bean stem and the proportion leaving the system when presented different second-instar conspecific larvae

and aphid densities were compared using Genstat to carry out a Generalised Linear Model analysis for a Binomial Distribution using a Logit link.

2.4. Mutual interference between E. balteatus larvae

Experiments were performed in similar Petri dishes. A third-instar larva of *E. balteatus* starved for 2 hours was placed in the middle of the Petri dish and was offered 2, 8, 16 or 32 second-instar larvae of *E. balteatus*. In addition each third-instar larva was provided with 40 similarly sized aphids. This gave us 4 treatments. There were 10 replicates of each treatment. A piece of broad bean plant was added to each dish as a source of food for the aphids and humidity for the larvae.

Hoverfly larvae were observed continuously during 30 minutes and the following behaviours recorded: (a) movements, (b) contacts with larvae or aphids, (c) attacks on larvae. Numbers of aphids left at the end of each replicate were recorded. This allowed us to determine the searching efficiency (aphids captured/ unit time/ hoverfly larva) of *E. balteatus* larvae. To determine if there was some mutual interference between larvae of *E. balteatus*, the searching efficiency was plotted against larva density on logarithmic scales, and the resulting graph slope compared to a slope of unity $b = 1$. Another statistical test confirming the reality of interference consisted in plotting Y against the log density of predators, with $Y = \log \{ \log (\text{initial aphid numbers} / \text{final aphid numbers}) \}$. Regression analysis was used to determine if there is some mutual interference.

Results

1. The role of floral resource area in hoverfly efficacy

Lettuce sampling

A total of 9,257 hoverfly eggs and 798 larvae of *M. fasciatum* and *M. novaezealandiae* were recorded over the sampling period (Table 7.1). Other predators found were the brown lacewing *Micromus tasmaniae* (Walker) and the 11-spot ladybird *Coccinella undecimpunctata* (L.). Hoverfly species were the commonest predators recorded; they represented 88 % and 73 % of the total egg and larva numbers sampled, respectively.

They were followed by the lacewings and by ladybirds. Moreover, but to a lesser extend, two kinds of mummies were encountered in the lettuces, caused by the parasitoid *Aphidius* spp., and the entomopathogenic fungus *Erynia neoaphidis* (Remaudière & Hennebert). At least, a total of 51,745 lettuce aphids *Nasonovia ribisnigri* (Mosley) and 284 black bean aphid *Aphis craccivora* (Koch) were found through the sampling period.

TABLE 7.1

Mean numbers of insects sampled weekly in the lettuces between 16 February and 22 March 2006. Numbers in brackets are the percentages of predator eggs and larva.

Treatment	Hoverfly		Lacewing		Ladybird		Mummies		Aphids	
	eggs	larvae	eggs	larvae	eggs	larvae	<i>Aphidius</i>	<i>E. neoaphidis</i>	<i>N. ribisnigri</i>	<i>A. craccivora</i>
12m×12m	19.87 (91.2)	1.24 (71.2)	1.35 (6.2)	0.48 (27.5)	0.56 (2.6)	0.022 (1.3)	0.29	0.63	74.49	0.37
3m×3m	9.71 (85.6)	0.975 (74.4)	1.37 (12.1)	0.27 (20.3)	0.26 (2.3)	0.069 (5.3)	0.57	0.89	66.13	0.39
Control	10.13 (85.5)	1.14 (75.4)	1.31 (11.1)	0.37 (24.3)	0.41 (3.4)	0.005 (0.3)	0.097	0.51	77.41	0.32
Total	39.71 (88.3)	3.36 (73.5)	4.03 (9)	1.12 (24.4)	1.23 (2.7)	0.096 (2.1)	0.96	2.03	218.03	1.08

Differences between treatments

Hoverflies laid significantly more eggs in the 12 m × 12 m plots than in the 3 m × 3 m and control ($F = 14.798$; $df = 2, 686$; $P = 0.000$; Fig. 7.2). More syrphid larvae were recorded in the 12 m × 12 m plots than in the two others plots, however differences were not significant ($F = 1.725$; $df = 2, 686$; $P = 0.179$; Fig. 7.3). Results showed that more aphids were sampled in the control plots but there were no significant differences ($F = 0.870$; $df = 2, 686$; $P = 0.419$; Fig. 7.4). There were highly significant interactions between egg, larva or aphid number and sampling dates ($P = 0.000$) and between egg, larva or aphid number and plots ($P = 0.000$).

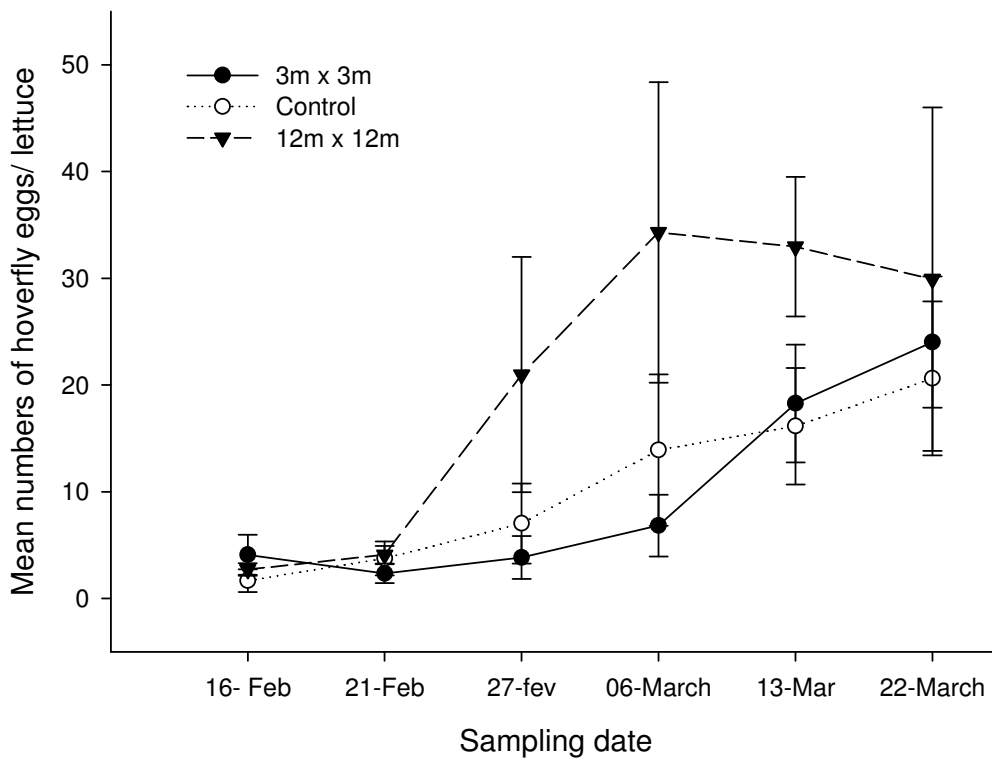


Figure 7.2. Mean numbers of hoverfly eggs (\pm SE) recorded per lettuce in each treatment between 16 February and 22 March 2006.

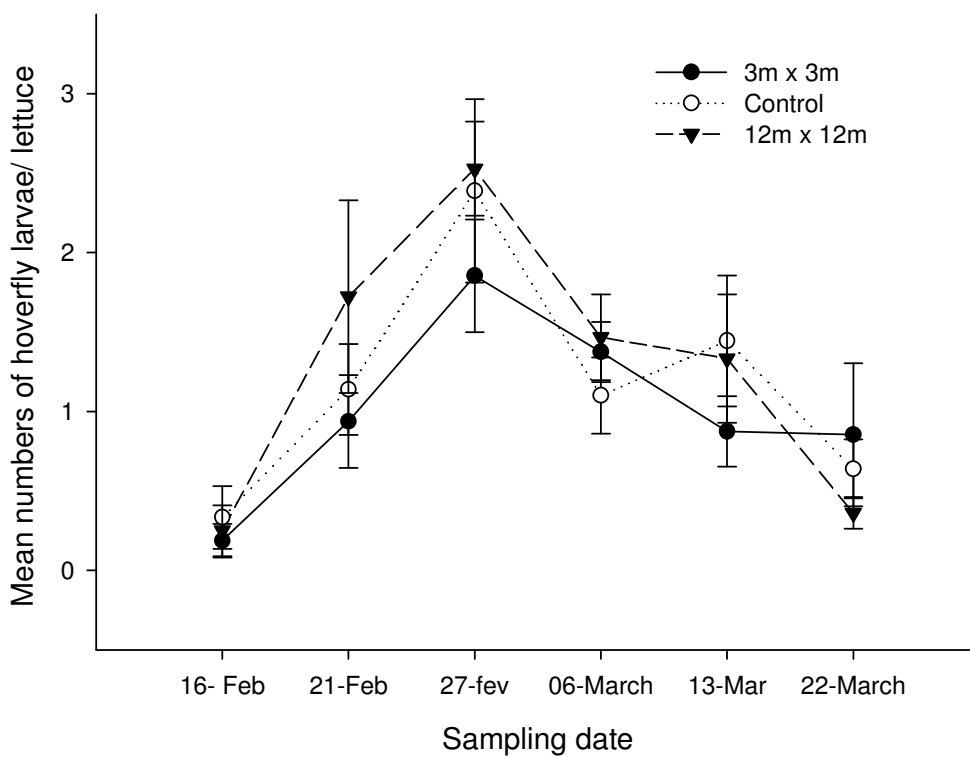


Figure 7.3. Mean numbers of hoverfly larvae (\pm SE) recorded per lettuce in each treatment between 16 February and 22 March 2006.

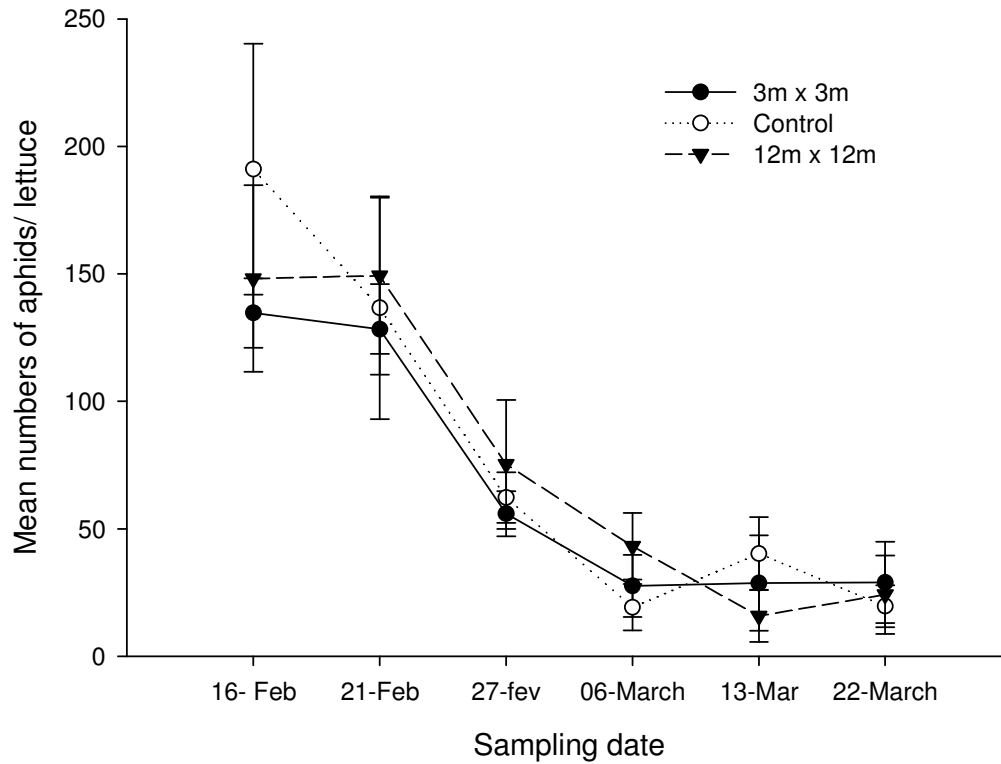


Figure 7.4 Mean aphid numbers recorded per lettuce (\pm SE) in each treatment between 16 February and 22 March 2006.

TABLE 7.2

Ratios of hoverfly: aphids observed in the lettuces for each treatment.

Treatment	Ratio of hoverfly : aphid	
	Eggs	Larvae
12m×12m	1: 3.8	1: 60.4
3m×3m	1: 6.9	1: 68.2
Control	1: 7.7	1: 68.2

The ratio of predator eggs to aphids was low in the three treatments and ranged from 1:3.8 in the 12 m \times 12 m plots to 1:7.7 in the control ones (Table 7.2). Then the larvae: aphid ratios were much lower and differences between treatments negligible, as they ranged from 1:60.4 in the 12 m \times 12 m plots to 1:68.2 in the control.

2. *Episyrphus balteatus* larval behaviour

2.1. Preliminary experiment: Is hoverfly fecundity high enough to reduce aphid populations?

TABLE 7.3

Mean numbers of aphids per plant recorded from aphids and first-instar hoverfly larvae released at initial ratios 1: 25, 1: 8.3 and 1:16.6 to larva pupations.

Days after release	Mean aphid number per plant					
	Ratio 1: 25		Ratio 1: 8.3		Ratio 1: 16.6	
	Control	Experiment	Control	Experiment	Control	Experiment
0	10	10	10	10	20	20
3	46.3	39.5	27.7	7.3	50.8	40.6
5	64.2	38.6	35.7	1.7	60	32.5
7	82.3	37.8	46.9	0.6	70.3	28.32
9	166.5	50.6	76.7	1.04	101.2	36.4
11	296.5	127	123.2	2.15	165.4	55.6
14			226.1	7.7	210.5	127.3

When *E. balteatus* larvae and *A. pisum* were released in predator-prey ratio of 1:25, hoverfly larvae failed in controlling aphid populations. Aphid abundance increased steadily for the first three days and remained constant until day 7, with a mean of 37.8 aphids per plant, but then increased to reach on day 11 a mean of 127 aphids (Table 7.3; Fig. 7.5).

At ratio 1:8.3 the aphid density was greatly reduced by syrphid larvae and after 5 days aphid colonies had almost disappeared (Fig. 7.6). However data given in Table 7.3 shows that after day 7 aphid populations started to increase again to reach on day 14 a mean of 7.7 aphids per plant. When initial aphid density was increased to 20, aphid colonies hardly established in both treatments. Aphid density was relatively low until day 7 but after this date it increased rapidly to reach on day 14 a mean of 127.3 aphids per plant (Fig. 7.7).

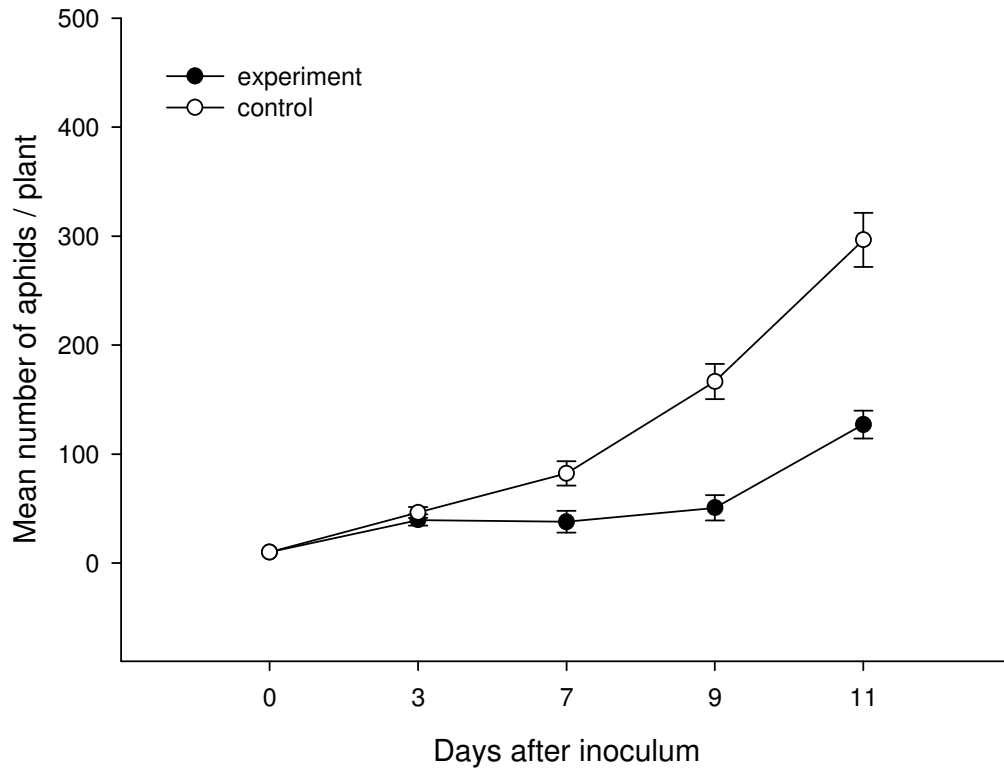


Figure 7.5. Number of aphids per plant against time (days) since inoculation. Initial ratio 1:25.

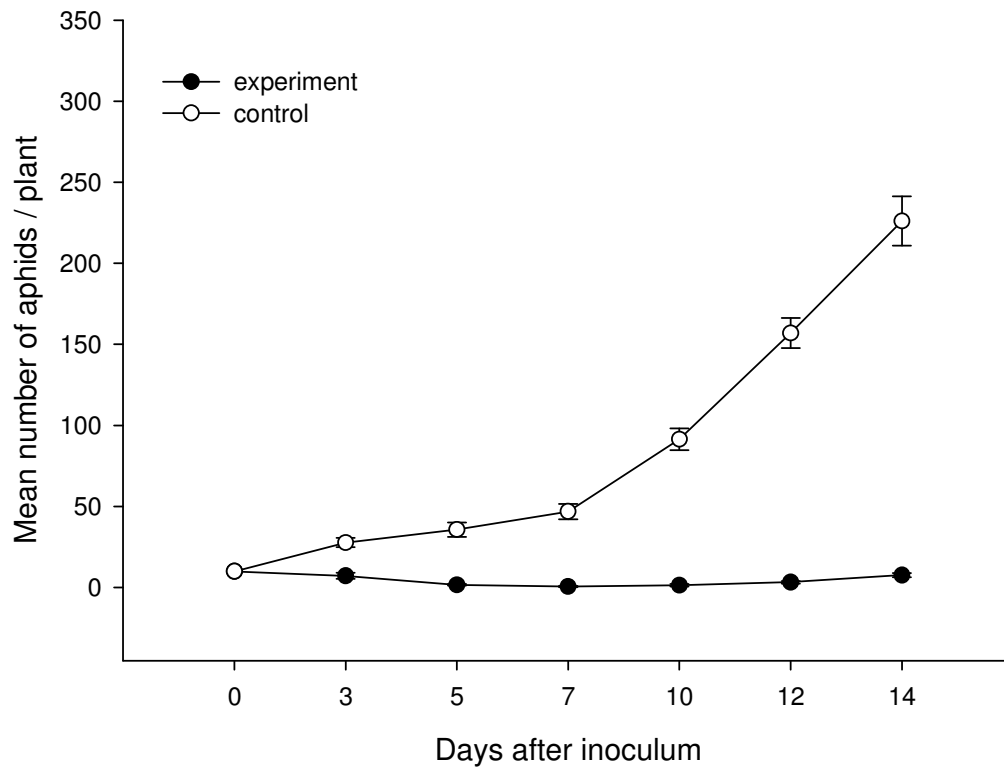


Figure 7.6. Number of aphids per plant against time (days) since inoculation. Initial ratio 1:8.3.

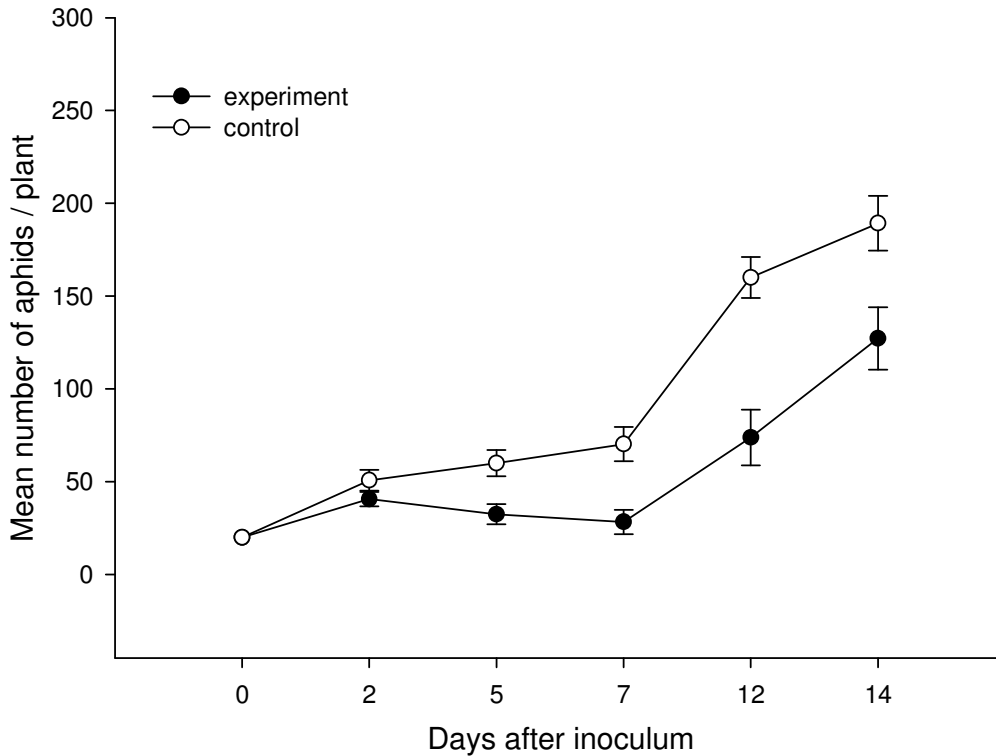


Figure 7.7. Number of aphids per plant against time (days) since inoculation. Initial ratio 1:16.6.

However, each trial had a single replicate; apparent differences between the control and experimental treatments were confounded with different treatments and times when the experiment was carried out. Thus, aphid numbers on the control plants for the ratio 1:8.3 were much lower than those for the other treatments and whether the low values observed for the treatment at ratio 1:8.3 were due to some environmental or subject material effect (temperature, age of aphids) rather than to the actual treatment could not be determined.

Nevertheless, although similar patterns were perceived for the three experiments, it seemed that the best results were observed when aphids and larvae were released at ratio 1:8.3. Treatments in which we introduced 10 aphids/ 0.4 larvae (ratio 1:25) and 20 aphids/ 1.2 larvae (ratio 1:16.6) gave similar results. While increasing the initial number of aphids inoculated to 20 per plant, the aim was to determine if this extension had a strong impact on larval efficacy. Some differences appeared in the first days of the experiment but then larvae controlled aphid populations in the same way.

2.2. Larval movements in time on broad bean plants

It appeared that time highly affected the proportion of larvae moving ($\chi^2 = 16.46$; $df = 5$; $P < 0.01$) (Fig. 7.8). In the first hours of the experiment, time for the larvae to settle on the plant, half of them were moving while the other half were not. Then the proportion of active larvae increased throughout the progress of the experiment and this was related to a decrease of aphid numbers. The last measurement data are omitted as larvae had started to pupate.

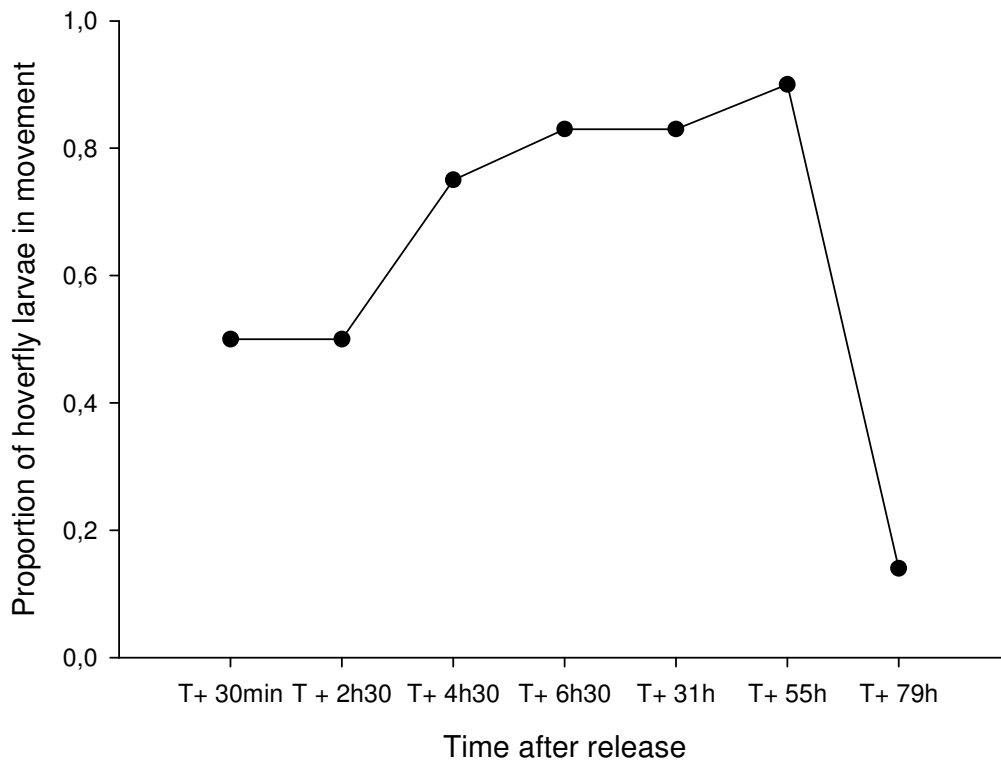


Figure 7.8. Proportion of second-instar larvae of *E. balteatus* moving on the broad bean plants against time at predator: prey ratio 1:8.3.

Active larvae were classified according to their movement in the system, this gave us three classes: larvae moving on the same plant, larvae moving between plants in the same pot and larvae moving between pots and/or leaving the system (Fig. 7.9). Larvae moved differently according to the experiment length of time. Thus the higher proportion of larvae moving on the same plant was observed between 30 min and 2 h 30, when aphid density was approximately 8-10 per plant. Then a maximum number of larvae circulating between plants, but still in the same pot, were observed after

6 h 30, which corresponded to an aphid density of 6-7 per plant. At least larvae leaving the pot were observed after 55 h, when aphid density was no more than 1.6 per plant.

Differences between the proportions of larvae in each category against time were significant ($\chi^2 = 41.54$; $df = 10$; $P < 0.01$). When aphid density was high larvae remained closed to the place they emerged. When aphid number decreased, larvae started to leave the plant but did not move far as they remained in the same pot. When prey were scarce, then they moved more and left the system.

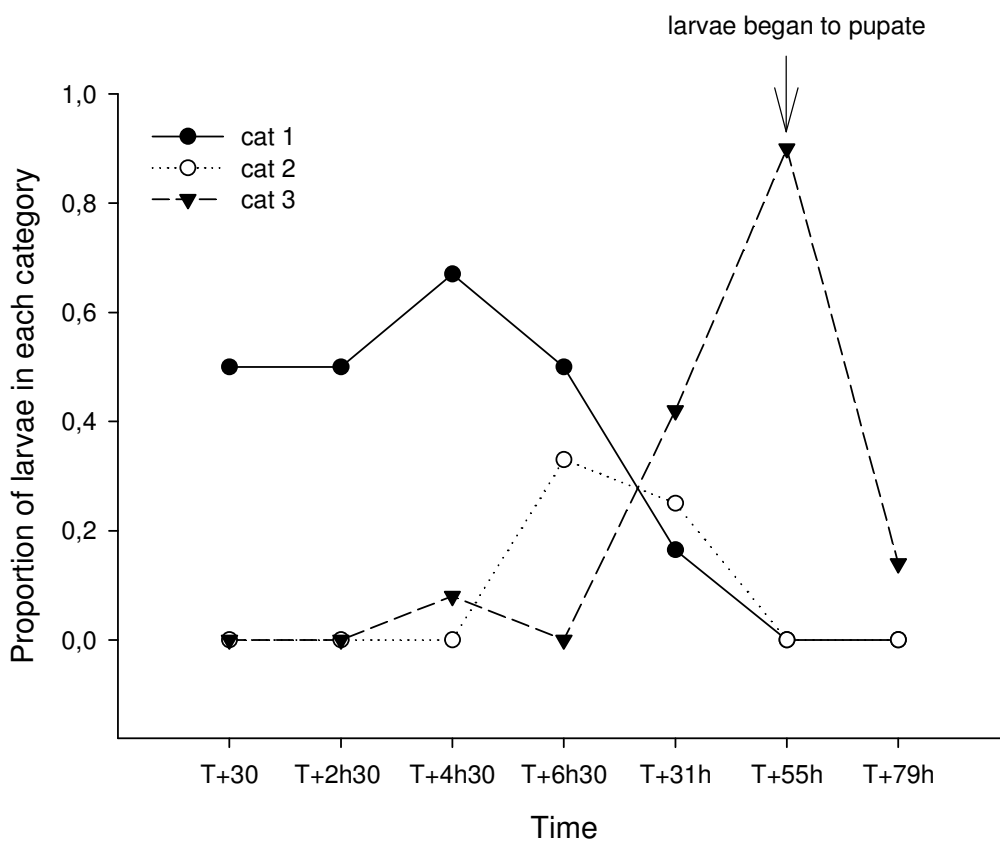


Figure 7.9. Moving of second-instar larvae of *E. balteatus* in the system against time at predator: prey ratio 1:8.3. Proportion of larvae corresponding to each category: Cat 1: moving on the same plant. Cat 2: moving between plants in the same pot. Cat 3: leaving the pot (includes moving between pots and leaving the system).

2.3. Effects of aphid and conspecific larvae density on the behaviour of hoverfly larvae in Petri dishes

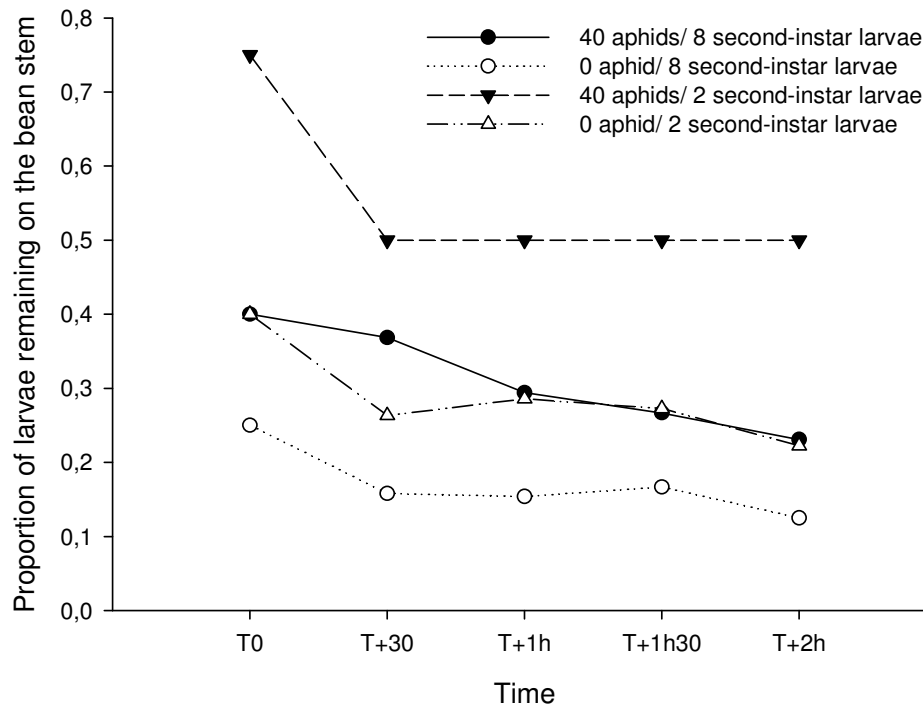


Figure 7.10. Proportion of third-instar larvae of *E. balteatus* remaining on the bean stem when presented different second-instar conspecific larvae and aphid densities.

The third-instar larvae remaining longer on the bean stem, where most of the aphids remained, were found in Petri dishes with 40 aphids and 2 second-instar larvae, which corresponded to the predator: prey ratio of 1:4.44 (Fig. 7.10). Then this tendency was observed in treatment with 40 aphids and 8 second-instar larvae (ratio 1:13.33). In treatment with 2 second-instar larvae only, third-instar larvae left the stem more rapidly, and even more when they were provided with 8 second-instar larvae only.

However, difference between the treatments in proportion of larvae still on the stem was observed only at Time 0. The proportion of third-instar larvae remaining on the stem in Petri dishes with 40 aphids and 2 second-instar larvae was significantly greater than the proportions for the other treatments ($F = 3.76$; $df = 3, 76$; $P = 0.01$). There was no evidence of differences between the other three treatments.

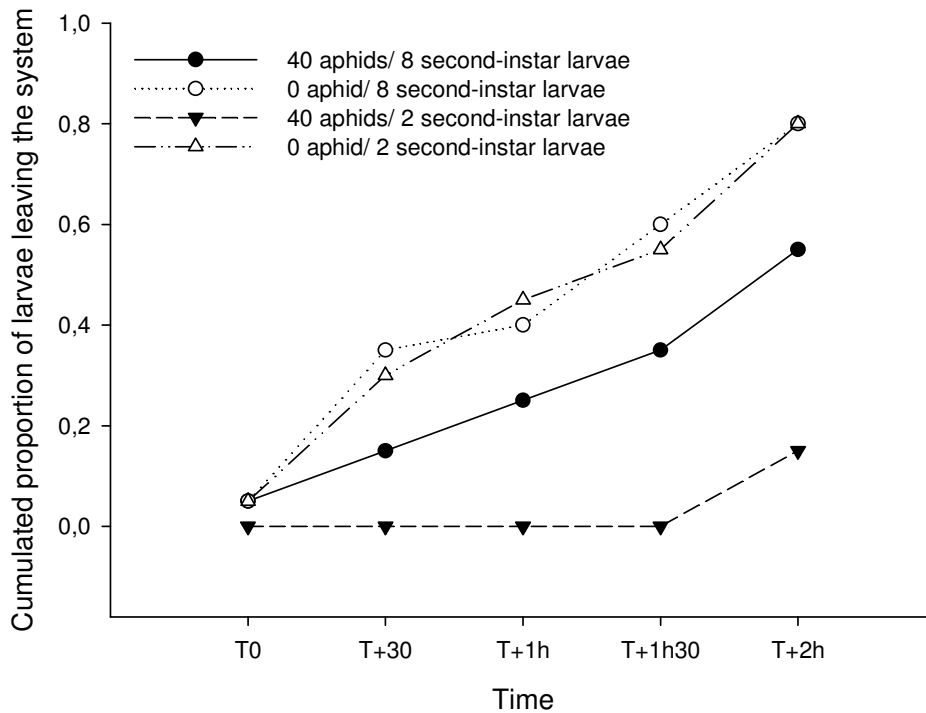


Figure 7.11. Cumulated proportions of third-instar larvae of *E. balteatus* leaving the system when presented different second-instar conspecific larvae and aphid densities.

Episyrphus balteatus larvae remaining longer in the system were those provided with 40 aphids and only 2 second-instar larvae (Fig.7.11). Differences observed with larvae provided with 40 aphids and 8 second-instar larvae can be explained by the fact that more larvae in the system ate the aphids available quicker and then moved, looking for more prey. However curves corresponding to larvae supplied with 2 or 8 second-instar larvae are very similar. One factor that would explain larval leaving is hunger. One other would be encounter rate with conspecific larvae, which could uncouple some cannibalism or mutual interference. This hypothesis is supported by the differences observed previously between third-instar larvae provided with 2 or 8 second-instar larvae, and the fact that larvae confronted to 8 conspecifics left faster the 'presumed' food source.

Differences observed between each treatment were significant only at Time + 2 h ($F = 8.21$; $df = 3, 76$; $P < 0.001$). However, analysis for the times T + 30 min, T + 1 h and T + 1 h 30 was possible only for the three treatments: 40 aphids and 2 second-instar larvae, 0 aphid and 2 second-instar larvae, 0 aphid and 8 second-instar larvae because in

the last treatment (40 aphids and 8 second-instar larvae) none of the larvae had left the system so there was no variation and this treatment had to be excluded.

Cannibalism in E. balteatus

During those 2 hours of experiment, larvae never ate each other. In some replicates, dishes were left overnight in order to detect any cannibalism after a long diet period; for each treatment 17 dishes were kept (Table 7.4).

TABLE 7.4

Cannibalism of second-instar larvae by starved third-instar larvae of E. balteatus in the absence of aphids after 20 hours.

Density of second-instar <i>E. balteatus</i> larvae	No of replicates (No of larvae in total)	Number of victims
2	17 (34)	3
8	17 (136)	13

After 20 h during which syrphid larvae were food deprived, cannibalism did occur when third-instar larvae were kept in Petri dishes with 2 or 8 second-instar conspecifics.

2.4. Mutual interference between *E. balteatus* larvae

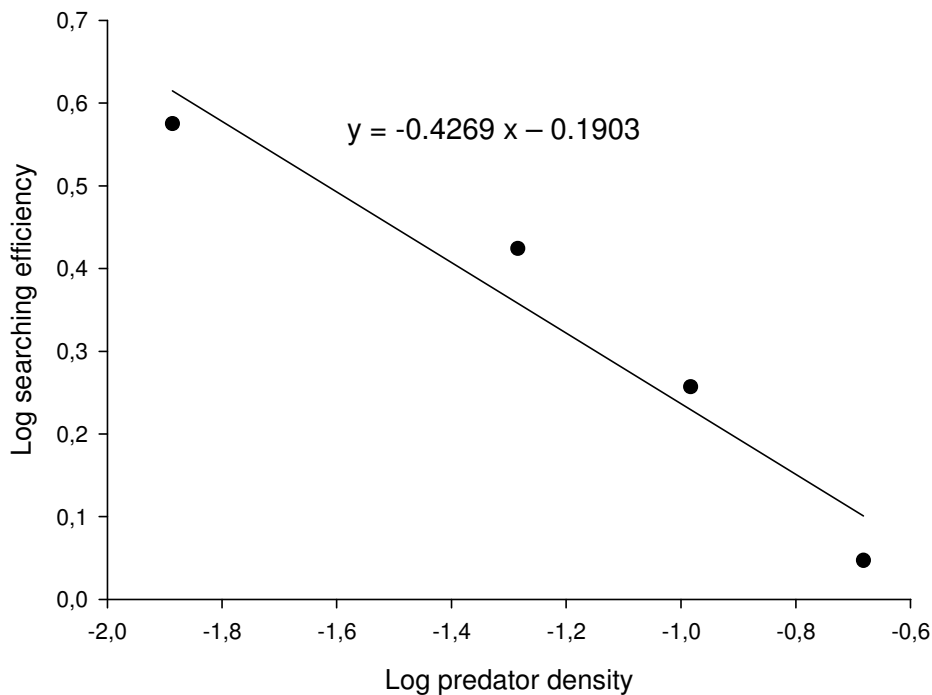


Figure 7.12. Searching efficiency (aphids captured/ unit time/ hoverfly larva) as a function of density of predators. $F= 47.01$; $df= 38$; $P<0.05$.

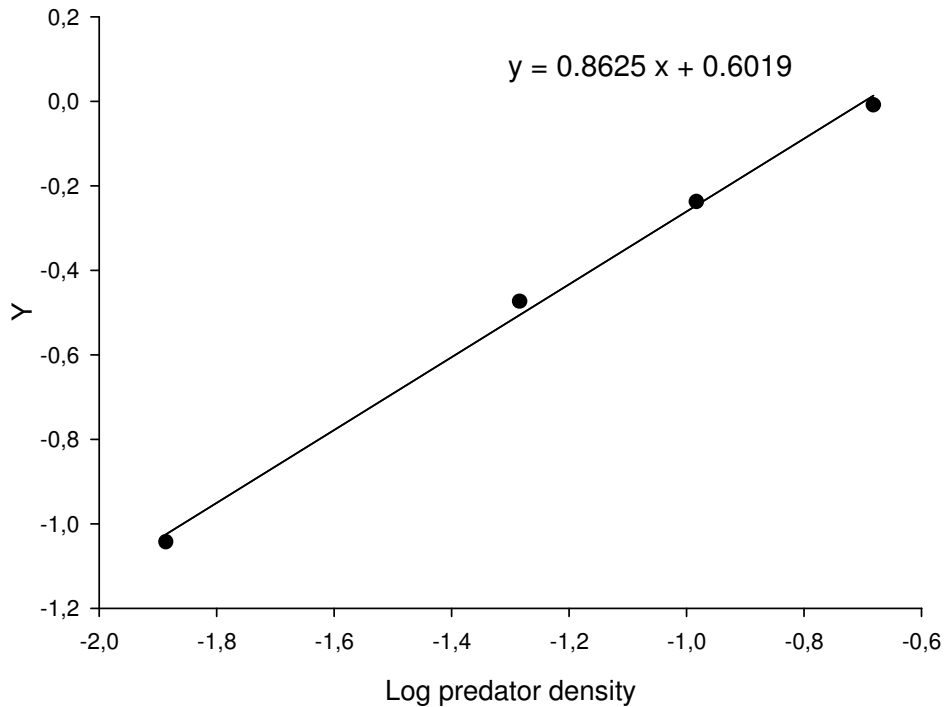


Figure 7.13. Statistical test confirming interference relationship for *E. balteatus*.
 $Y = \log \{ \log (\text{initial aphid numbers} / \text{final aphid numbers}) \}$.

Results of the regression analysis confirmed that there was mutual interference between the larvae of *E. balteatus* ($F = 3.42$; $df = 38$; $P < 0.05$). It seemed that searching efficiency of hoverfly larvae was a function of the number of hoverflies in the area (Fig. 7.12 & Fig. 7.13).

Discussion

As hoverfly species were the commonest predators recorded, they represented 88 % and 73 % of the total predator egg and larva numbers sampled. That's why it can be assumed that the mean effect on aphids was caused by hoverflies more than other predators and parasitoids.

If the presence of buckwheat enhanced biocontrol of lettuce aphids by hoverfly larvae, a higher rate of oviposition and fewer pests in the 12 m × 12 m plots would be expected compared with the 3 m × 3 m and the control plots. More hoverfly eggs and larvae were sampled in the 12 m × 12 m plots, but more syrphid eggs and larvae were also found in the control plots compared with the 3 m × 3 m plots. However, buckwheat flowers of

the 3 m × 3 m plots hardly grew in most of the replicates and were poorly attractive to nectar and pollen feeding insects. This can explain results obtained in the 3 m × 3 m and the control plots, which should all be considered as control in this study. Differences in the numbers of eggs laid between treatments were significant however this was not the case for hoverfly larvae.

The greatest numbers of aphids were recorded in the control plots, but more aphids were also found in the 12 m × 12 m than in 3 m × 3 m plots; however differences between treatments were not significant. As more hoverfly eggs and larvae were found in the 12 m × 12 m plots, one would expect fewer aphids in this treatment compared with the two others but this was not the case.

Although we observed ratios of hoverfly eggs to aphids between 1:3.8 and 1:7.7, then ratios of larvae to aphids ranged from 1:60.4 to 1:68.2. Number of eggs in the 12 m × 12 m plots was almost 16 times greater than the following number of larvae sampled, while in the control plots, number of eggs was 9 times greater. As recorded Chambers (1986), at constant temperature 21°C ± 2 under a long photoperiod, the hatch rate was 45.1 %. So under natural conditions it is likely that a limited number of eggs hatched. Moreover, typically, hoverfly larvae are not active during daylight hours unless hungry and some species rest lower down the plant, away from aphid colonies (Chambers, 1986).

The results of our experiment imply that the density of hoverfly eggs and larvae is not inversely correlated to the density of aphids present in the crop.

Hoverflies laid an increasing number of eggs in the 12 m × 12 m plots until the 6th of March and then it decreased, while in the 3 m × 3 m and control plots, where hoverflies laid significantly fewer eggs, curves increased throughout the experiment. This difference may be explained by comparing New Zealand hoverflies with European, of which some species were observed avoiding aphid colonies when syrphid eggs were present (Scholz & Poehling, 2000). Larvae of *E. balteatus* attack syrphid eggs and smaller syrphid larvae (Branquart *et al.*, 1997), and conspecific larvae also act as an oviposition deterrent for *Syrphus nitidicollis* (Meigen) (Hemptinne *et al.*, 1993). The first weeks of the experiment when syrphid eggs and larvae were relatively not

abundant, their presence did not inhibit adult oviposition; it is also possible that the New Zealand species are less selective than *E. balteatus* and they stop ovipositing when a threshold of eggs already laid is reached. While some laboratory experiments and field studies suggest that positive density-dependent oviposition occurs in *E. balteatus* (Chandler, 1968b; Geusen-Pfister, 1987; Tenhumberg & Poehling, 1991; Bargaen *et al.*, 1998; Scholz & Poehling, 2000), field observations have also suggested that female syrphids avoid plants with large or ageing colonies (Kan & Sasakawa, 1986; Kan, 1988a, b, 1989). In the decision-making process, there may be a trade-off point at which a female syrphid will no longer oviposit near an aphid colony (Sutherland *et al.*, 2001b). Experiments with *M. fasciatum* and *M. novaezealandiae* on oviposition behaviour have never been done and such information is missing.

It is well established that hoverflies feeding on floral resource subsidies remain in the local area longer (Molthan & Ruppert, 1988; Sengonça & Frings, 1988; Lövei *et al.*, 1992; MacLeod 1992; White *et al.*, 1994, Morris & Li, 2000), and in the present work it appears to be the case as female hoverflies laid more eggs where floral resources were more abundant. This result contrasts with those of Chandler (1968a) who found no difference in oviposition in small replicated plots of Brussels sprouts between those with buckets of flowers added and those without. The hypothesis suggested in our introduction, that in previous experiments resource subsidies were not sown in quantities large enough to attract and make hoverflies remaining in the local area is supported by our results.

Contrary to expectations, higher oviposition rates did not lead to improved aphid population suppression. In field cage experiments, hoverfly larvae have proved to be efficient biological control agents of pest aphids (Tenhumberg, 1995; Tenhumberg & Poehling, 1991). Results of this study may indicate that larvae under field conditions are not as efficient as predicted by field cage studies. Hoverflies might be limited in the field by other predators. Hindayana *et al.* (2001) showed that eggs, first- and second-instar larvae of *E. balteatus* were highly susceptible to intraguild predation by the ladybird *Coccinella septempunctata* (L.) and the common green lacewing *Chrysoperla carnea* (Stephens). Moreover, Branquart *et al.* (1997) showed that cannibalism occurred frequently in a laboratory population of *E. balteatus* and that mainly eggs were eaten by third instar larva. That is why the presence of other predators was recorded in this study

but the relatively few numbers of the brown lacewing *M. tasmaniae* and the ladybird *C. undecimpunctata* sampled made us conclude that hoverfly eggs and larvae were more confronted by intraguild predation than interspecific.

Finally it can be concluded that flower area highly influenced oviposition behaviour of female hoverflies, however more eggs did not lead to sufficient numbers of larvae to improve aphid population suppression.

In lettuce fields where numbers of aphids and larvae were so high lots of larvae disappeared. The same trend was observed in the greenhouse experiment on bean plants in pots. Even when female fecundity was supposed to be high (ratio 1:8.3) larvae did not succeed to control aphid populations. Seven days after the beginning of the experiment, aphid colonies had almost disappeared: 29 plants were cleaned of aphids, 10 plants only had 1 aphid and the last one had 14 aphids, while on the control we counted a mean of 46.9 aphids per plant. However those remaining aphids were not consumed and aphid populations increased again, to reach on day 14 (when the experiment was stopped) a mean of 7.7 aphids per plant. At the end of the experiment, plants were dissected to find either alive, dead larvae or pupae. Of the 48 larvae introduced at the beginning of the experiment, only 4 third-instar larvae, 3 second-instars, 3 dead larvae and 1 pupa were found on day 14. We supposed that this loss had several causes: the study of first-instar larvae which were easy to lose because of their small size; the larvae could have left the system in search of prey, motivated by hunger; the larvae could have left the system because they avoided each other. Similar loss has been observed in others experiments; Chambers (1986) who studied *M. corollae* feeding on *A. gossypii* explained this loss was due to searching during which some larvae moved down the plant stem and onto the soil surface and pot rim. Larval loss was significantly and inversely related to aphid density; at higher aphid densities fewer larvae left the plant in search of prey. In his study, control of aphids by *M. corollae* was achieved rapidly, and one day old larvae prevented aphid increase unless the predator: prey ratio fell below 1:15. However, predation pressure was sustained for only a few days. Bondarenko & Asyakin (1981) found the same dispersal tendency at low aphid density in experiments using hoverfly larvae on single leaves of cucumber, suggested that predatory control would be more stable in a plant stand where larvae can redistribute between plants and thus help compensate for any short-term absence of

ovipositing females. In our study, experiments were conducted at low predator: prey ratios and *E. balteatus* larvae had the possibility to move between plants, but they still did not consume the last aphids remaining.

Few studies have been done on larval efficiencies in relation to aphid densities. Scott & Barlow (1986) investigated foraging efficiency of *M. corollae* larvae by varying the number of aphids offered to larvae and the area of the plants (pea) on which prey were placed. They showed that consumption of aphids by syrphid larvae increased with the number of prey available and decreasing plant size. A larger plant surface area may change the spatial heterogeneity or patchiness of the prey distribution, which became harder to find, thus affecting searching success. These observations could explain why larvae of *E. balteatus*, *M. fasciatum* and *M. novaezelandiae* failed in controlling aphid populations in the previous experiments on bean and lettuce plants. In the former larvae may not have found the last aphids remaining; in lettuces, plant surface area grew throughout the experiment making aphid populations harder to be detected by larvae.

We showed that syrphid larvae could initiate a decline in aphid numbers, at the ratio 1:8.3 that would be effective; however this control did not last since aphid populations started to increase again. Our results are very different from some others observed in the field. Tenhumberg and Poehling (1991) released second-instar larvae of *E. balteatus* in 2×5 m² cages in several predator-prey-ratios. Even at densities 1:245, aphids were nearly complete eliminated. However, they stopped the experiment after 6 days, while it would have been interesting to see what happened latter.

Experiments on broad bean plants in the laboratory showed us that while aphid numbers declined, larvae became more active and moved to plants in the vicinity or left the system. When numbers of larvae were higher, larvae left the system even more rapidly and it was showed that this behaviour was caused by two main factors: hunger, but also avoidance of conspecific larvae.

Biological control strategies against aphids are aimed at achieving high densities of natural enemies at the beginning of the development of pest populations. It is established that the introduction of floral resource subsidies within crops increases oviposition behaviour of female hoverflies, but it is also increasingly likely that high

densities of larvae will facilitate encounters while searching for prey, which in turn may lead to an increased tendency toward dispersal (Hassel, 1978). Our findings highlight the conclusion of Hindayana *et al.* (2001) that the low cannibalism rates observed suggest that cannibalism in *E. balteatus* should not be important under field or greenhouse conditions. The incidence of cannibalism in the field has never been measured (Branquart *et al.*, 1997). The results of our experiments in Petri dishes showed that hoverfly larvae missing in the system are due to mutual interference rather than eating each other. Increased densities of larvae led to increased emigration rates and caused a decrease in feeding efficiency. Surprisingly, predators are limiting themselves, instead of responding to prey density.

These observations might explain the ‘buy-futures’ ovipositional tactic of females in the field. To maximize their fitness, females should avoid ovipositing where their offspring are likely to starve, such as in aphid colonies that are declining in abundance when alatae are declining in mass. Starvation can also result from competition between larvae for food. Field observations indicate that females of several species of syrphid avoid ovipositing in old aphid colonies, which they recognize by the presence of winged aphids (Kan, 1988a, 1988b). It has also been established that they avoid aphid colonies with syrphid eggs, as long as aphid colonies without eggs were present (Kan, 1988a; Hemptinne *et al.*, 1993; Hindayana *et al.*, 1999; Scholz & Poehling, 2000). Several characteristics of the reproductive strategy of syrphids may reduce the incidence of mutual interference.

In summing up, results of these experiments showed that flower area highly influenced oviposition behaviour of female hoverflies; however more eggs did not lead to more larvae and did not translate into a better aphid population suppression. From the experiments in the greenhouse it can be assumed that as lettuces were separated from each other, larvae could not redistribute between plants and could get lost; moreover their surface area grew throughout the experiment, making aphid populations harder to be detected by larvae. However, following larvae in the laboratory, it was demonstrated that the leaving rates from a system were related to their densities. Mutual interference was observed between hoverfly larvae, which led to a decline in searching efficiency.

Chapter 8

General discussion

Most attempts to manipulate habitats within agroecosystems to manage pest populations have been intuitive rather than based on sound science (Gurr *et al.*, 1998). Rather than carry on proceeding by trial and error, at present it seems important to focus on work done in the past to determine causes of those failures. Actually, it appears that the main theories from which the present techniques of biological control are elaborated do not always rest on a full knowledge of the agents' biology. These theories do not usually include the notion of 'fitness' of the biological control agents.

Of the major forms of biological control, conservation biological control has received the least amount of attention, until recent times (Ehler, 1998). Because of the importance of the ecology of the agents, such things as phenology, floral preferences and oviposition behaviour of the adults and prey preferences, searching efficiency and behaviour of the larvae, conservation biological control should be considered in all biological control programs (Landis *et al.*, 2000)

The present work aimed to study further the behaviour and ecology of one natural enemy group, the hoverflies, in order to understand the potential of these insects for biocontrol.

Although hoverflies have the potential to slow aphid population growth, in most of the cases observed, in the field but also in laboratory experiments, their activity has not been sufficient to reduce efficiently aphid populations. In order to determine the causes of failure observed using hoverflies in CBC programmes, it is important to focus on several aspects of their behaviour. On the one hand the foraging behaviour of females is well documented, but there are some conflicting data: while many authors have reported that positive density-dependent oviposition occurs (Chandler, 1968a; Geusen-Pfister, 1987; Tenhumberg & Poehling, 1991; Bargaen *et al.*, 1998; Scholz & Poehling, 2000), several studies suggest that female syrphids prefer smaller or younger aphid colonies for

oviposition (Kan & Sasakawa, 1986; Kan, 1988a, 1988b, 1989; Hemptinne *et al.*, 1993). Moreover, some work (Hindayana *et al.*, 2001; Branquart *et al.*, 1997) done on cannibalism and intraguild predation in hoverflies is consistent with the evolution of such tactics. On the other hand, apart from the predator: prey ratios required, studies on larval behaviour have been largely neglected so far. However, intra- and interspecific competition may be an important regulatory factor in predator performance so syrphid larvae may have developed strategies to increase foraging efficiency.

CBC by hoverflies involves agroecosystem manipulation through the introduction of floral resources that have the potential to attract and enhance the effectiveness of female hoverflies. Females are expected to lay eggs within the adjacent crop with the emerging larvae consuming aphids. Thus, this work aimed at making progress in the knowledge on the effects of floral resources on female hoverfly 'fitness' (1) in the laboratory through the study of adult nutrition and effects of pollen and nectar on fecundity and longevity; (2) in the field through movement and oviposition behaviour. Moreover, larval predatory efficacy was analysed further.

Expected levels of success of hoverflies as biological control agents

As discussed in Chapter 7, there exists a hierarchy of outcomes that researchers hope to achieve when conducting CBC investigations (Gurr *et al.*, 2003). Demonstrating an increase in hoverfly abundance in the presence of floral resources is the first step in achieving the benefits of planting flowers to enhance biological control. In Chapter 5, but also in Chapters 6 and 7, *M. fasciatum* and *M. novaezelandiae* abundance was increased when flowering phacelia and/or buckwheat were added to broccoli, carrot and lettuce systems, thus the first step was achieved.

Evidence of an enhancement of natural enemy 'fitness' is considered as the second step and this was demonstrated in Chapter 4, where phacelia and buckwheat flowers increased the net reproductive rate of *E. balteatus* in laboratory experiments.

The third step, namely an increase in predation rate of the pest population, was demonstrated in Chapter 7. An increase in oviposition rate was observed where flowering buckwheat areas were larger.

It is logically expected that the fourth step should present some evidence of a reduction in the pest abundance. This was not achieved in the present study. Contrary to expectations, higher oviposition rates did not lead to improved aphid population suppression. It appeared that more eggs did not lead to more larvae and did not translate into better aphid population suppression.

The fifth step, reducing pest populations below an economic threshold was not measured directly; however, the results of Chapter 7 suggest that pest populations could not be decreased below the relevant economic threshold. Moreover, although the evaluation of predation rates and pest abundance was not the aim of the Chapter 6, the experiment was set up on a commercial field provided by a farmer which had to spray the insecticide neem over the crop, thus giving evidence that hoverflies failed to reduce aphid populations below economic threshold, at least at the stage that the crop was sprayed.

The work presented in this PhD contributes to improve knowledge of hoverfly efficacy and provides some explanations for the lack of success that has been so often observed. The results constitute a contribution to fundamental knowledge on the predator-prey relation through the study of oviposition behaviour of female hoverflies among aphid colonies and the larval behaviour in terms of movements in aphid colonies and effects of larvae on conspecifics. These results are also of practical importance for the development of CBC programmes. An appropriate choice of flowers depends on hoverfly preference and will affect female fitness; the location of the floral resources in agroecosystem will depend on female dispersal after they feed on pollen and nectar and the extent to which oviposition rate is enhanced.

Contribution to fundamental knowledge

By definition, an efficient biological control agent must give a sufficient reproductive or aggregative numerical response to inflict a mortality rate that is greater than the growth rate of the prey (Beddington *et al.*, 1978). In general predatory efficacy depends on its consumption rate. However, oviposition behaviour of females can induce a numerical response of equal or greater importance. Efficacy of hoverfly larvae also depends on how the adults lay eggs among aphid colonies. The latter are temporary food sources, which are patchily distributed and have a short life span (Dixon, 1985), so females must develop special adaptations to be able to take advantage of them. Females should avoid ovipositing where their offspring are likely to starve and this could happen when too many eggs are laid in a colony or too late in the development of the colony, i.e., when the aphids are beginning to disperse. There is evidence that syrphids have evolved behavioural mechanisms that have enabled them to forage in a way that is consistent with optimal foraging theory, avoiding colonies that are already exploited and/or will not last long enough to support the development of their larvae (Kan, 1988a; Hemptinne *et al.*, 1993; Hindayana *et al.*, 1999; Scholz & Poehling, 2000).

Although the addition of floral resources has been widely shown to enhance hoverfly activity and local abundance, a successful control of aphid colonies has been rarely achieved as a result. The oviposition behaviour of female and foraging behaviour of larval hoverflies can partly explain the lack of success observed when implementing CBC programmes.

Oviposition behaviour of female hoverflies

There is good evidence, from laboratory experiments and field studies to suggest that a positive density-dependent oviposition occurs in hoverfly species such as *E. balteatus*. However, field observations have also suggested that female syrphids avoid large or ageing aphid colonies, especially those with alate adults (Kan, 1988a, 1988b). In the laboratory, female *E. balteatus* were not only able to adapt their oviposition in regard to aphid density but also to detect the presence of conspecific eggs and therefore oviposit less often if conspecific eggs are present (Scholz & Poehling, 2000). Conspecific larvae also act as an oviposition deterrent for *S. nitidicollis* (Hemptinne *et al.*, 1993). However,

it has also been reported that the presence of conspecific larvae does not influence the oviposition rates of *E. balteatus* (Chandler, 1968a; Bargaen *et al.*, 1998). The results in Chapter 7 suggest that hoverfly activity and oviposition behaviour were enhanced by the addition of floral resource subsidies but only to a certain extent. In fact, females laid an increasing number of eggs in the plots with larger floral area during the first month of the experiment, when aphid colonies were still small and young, then it decreased, supporting the 'buy-futures' ovipositional tactic (Kan, 1988a, 1988b; Sutherland *et al.*, 2001a). On the other hand, in the control and the small floral area, where numbers of eggs laid were significantly fewer, oviposition behaviour increased until the end of the experiment, and supported the positive density-dependent oviposition tactic. In the decision-making process, there may be a trade-off point at which a female syrphid will no longer oviposit near an aphid colony.

Foraging behaviour of larval hoverflies

A better understanding of larval behaviour might explain the foraging behaviour of female hoverflies. Instead of working with several floral resource areas, several densities of syrphid larvae were compared: systems were set up in order to obtain a stronger aggregative response. The last experiments presented in this thesis demonstrated that increased densities of larvae in the system would lead to increased emigration rates of these larvae and cause a decrease in aphid mortality rate. Mutual interference was observed between hoverfly larvae, limiting their response to prey density.

Supporting those findings, it is interesting to note that other aphidophagous insects have developed parallel mechanisms to overcome the same constraints. For example, females of some species of aphidophagous ladybirds refrain from ovipositing in patches where there are few aphids (Dixon, 1959) and conspecific larvae are present (Hemptinne *et al.*, 1992, 2001; Doumbia *et al.*, 1998) by responding to a species-specific oviposition deterring pheromone in the tracks of larvae. This is adaptive as larval cannibalism is a major threat to egg survival. The impact of an oviposition-deterring pheromone on the reproductive behaviour of insects has also been described for many species of chrysopids (Ruzicka, 1996, 1997 & 1998) and for the cecidomyiid fly *Aphidoletes aphidimyza* (Rondani) (Ruzicka & Havelka, 1998).

Concerning larval behaviour, it worth stressing that hoverfly larvae have a rather limited dispersal ability (Chandler, 1969; Sadeghi & Gilbert, 2000; Gilbert, 2005). As was demonstrated by Scott & Barlow (1986), a larger plant surface area may change the spatial heterogeneity or patchiness of the prey, which become harder to find, thus affecting the predator's searching success. Considering the lettuce experiment (Chapter 7), plants were initially spaced at 45 cm to restrict larval distribution between plants. Moreover, the lettuce plant surface area grew throughout the experiment, making aphid populations harder to be detected by larvae. If in addition mutual interference occurred, it is likely that hoverfly larvae disappeared as they dispersed.

Practical implications and future research

The effective use of syrphids in pest management would depend, amongst other things, on a better understanding of how they utilize patches that provide floral resources in terms of (1) adult hoverfly floral preferences and potential of floral resources in enhancing hoverfly fitness, in order to determine which plant species should be employed; (2) how far an adult syrphid will move after feeding and how far it will oviposit. Information of this type will provide guideline on how much resource is needed and where it should be deployed. This is theoretical, as, in practice, it is not easy to highlight all the factors needed to achieve effective biological control. Moreover, previous work in this thesis took place at the crop scale, while efficient control of aphids should consider the action of natural enemies at the landscape scale. That's why this discussion will develop other studies that are worth pursuing. Past research has indicated that the combined action of a range of natural enemies may be necessary for the successful natural control of aphid pests in arable field crops such as cereals (Wratten & Powell, 1991; Sunderland *et al.*, 1998). Given the observations made on foraging behaviour of females and mutual interference observed between larvae, and the lack of success of CBC by hoverflies in experiments set up at the crop scale, it is essential to assess the impact of insect predators and parasitoids in a unified way and test them at a commercial field scale.

Choice of floral resource subsidies

It is well established that there is a potential to attract hoverflies to floral resource subsidies, enhance their oviposition behaviour and better estimate their populations. To realise this, the provision of floral resource subsidies around or across fields should be done according to flowers that are highly visited and have the potential to increase hoverfly efficacy. This provision must be compatible with farming practices. However, although a wide range of flowering plants has been used for this purpose in agroecosystems (see Landis *et al.*, 2000), there has been relatively little research into the selection of the most appropriate plant species to employ (Landis *et al.*, 2000; Gurr *et al.*, 2004a) and further work should consider which floral resources are appropriate in particular conservation and pest management. Whether a well-studied single flower species, flower mixtures or weed management approach should be used remains to be resolved. While cultivating a well-studied single flower species ensures the conservation of a single targeted beneficial species and minimises the risk of non-target effects, such as inadvertently promoting populations of pests (Baggen *et al.*, 1999), higher-order predators or hyperparasitoids (Stephens *et al.*, 1998), the use of flower mixtures diversifies the resources available, which may cater for a greater diversity of natural enemies because of selective feeding on various floral components. This may also act to alleviate interactions between beneficial species for flowers, caused by direct contact, repellent scent cues or resource depletion (Pontin *et al.*, 2006).

The fact that some weed species were used selectively by hoverflies has implications for the production of guidelines for the management of field margins. Guidelines which encourage the growth of these species should favourably modify the agroecosystem for syrphids. Although the drilling of phacelia seeds around fields in spring has been estimated to be only one penny (UK) per meter of row (Wratten & van Emden, 1995), it may be economically impossible to sow single flower species, or even flower mixtures at the landscape scale. If flowering weeds could increase hoverfly performance to the same extent that phacelia did, it might be ecologically and economically more realistic to leave some fallow strips treated selectively to encourage the growth of the selected weed species acceptable by farmers and which are used by syrphids, rather than sow extra floral resource subsidies. However information on the nutritional value of the weeds is non-existent. Further work comparing, for example, phacelia or buckwheat and some of the weeds from which hoverflies and other beneficial insects were observed

feeding in this work would be useful to determine if the weeds can give such reproductive potential. Hoverflies such as *E. balteatus* are good flyers, thus landscape experiments should be conducted, with widely separated fields or farms as replicates, to detect the effects of the adoption of management strategies. Moreover, options compatible with farming practices need addressing further.

Raised questions and future research

Many questions deserving further attention to improve CBC remain unanswered. For example, do floral resource subsidies affect only hoverfly distribution or do they increase their abundance at the landscape scale? Very little work has investigated whether floral resources can enhance the fitness of natural enemies in the field (but see Vattala *et al.*, 2006) and this deserves further research, as many studies have assumed that enhanced fitness in the laboratory will lead to enhanced fitness in the field. In order to optimise biological control, the flower species cultivated should be based on laboratory and field assessments such as abundance, egg load and longevity (e.g., Hickman & Wratten, 1996; Irvin *et al.*, 1999; Tylianakis *et al.*, 2004; Berndt & Wratten, 2005).

The next question would be: how far do hoverflies move (and then oviposit) after feeding? It is difficult to demonstrate effects of flowers, probably because adult syrphids are highly mobile, and benefits acquired by pollen feeding (e.g., ovariole development) do not occur immediately. Moreover, nectar is a source of energy food and enhances dispersal (Bugg, 1992). Although many hoverflies feed on floral resource subsidies and have the ability to disperse within the crop, it does not follow that syrphids are able to travel between flowers and the crop in sufficient numbers and with a homogeneous distribution to improve pest suppression. Several authors have suggested that hoverflies may accumulate in florally rich field margins during their flower feeding phase, and subsequently fail to disperse into the crop effectively during their egg laying period (Lövei *et al.*, 1993; Harwood *et al.*, 1992,1994; White *et al.*, 1995; Wratten *et al.*, 2003). Sutherland *et al.* (2001b) reported that *E. balteatus* was associated with field margins rather than within-crop wildflower patches. Females were likely to utilize other resources that field margins offer, namely alternative aphid resources present on trees and other herbaceous hosts, shelter from predation, lekking

sites and suitable flight-paths. Finally they concluded that this apparent reluctance for *E. balteatus* to move into fields could make this species an unsuitable candidate for biological control *via* augmentation.

Studies done on parasitoids also suggest that floral resources do not increase general activity by wasps in adjacent crops. The general lack of an impact of floral resources on parasitism rates could be explained by between-replicate dispersal of wasps, in addition to other sugar sources being present in the system. While parasitoids feeding on sugars may remain in the local area longer, they may be limited by predators; it is also possible that they forage for hosts in distant host patches and no longer contribute to pest control in field adjacent to floral resources (Lavandero *et al.*, 2005; Lee *et al.*, 2006). The results obtained on hoverfly dispersal (Chapter 6) suggest that hoverflies dispersed far from the crop. This observation raises the problem that the floral resource subsidies could be too small compared with the crop area to exert a sufficient effect and make the flies remain in the local area. Future work could further examine the dispersal of female hoverflies from flowering plants at a landscape scale, as few females were caught in the crop itself even though high numbers were captured in the vicinity of the floral strips. Hoverflies are good flyers and have the ability to disperse widely from the flower sources, but maybe they disperse too widely to remain in the crop itself. However, is it possible to introduce a floral resource area large enough to modify hoverfly perception of the landscape? Given their dispersal ability, manipulation of the agroecosystem at a landscape scale should be envisaged. A greater understanding of spatial dynamics of female hoverflies could enable the further enhancement of the biological control of aphids.

Also, further work needs to focus on the impact of floral resource subsidies to both pest and natural enemy populations. Many pest species use the same floral resources that their predators and there is a potential risk of increase pest abundance and damage. For example, Winkler *et al.* (2005) demonstrated that the diamondback moth *P. xylostella* and its parasitoid *D. semiclausum* responded to and showed a prolonged life span on a broad range of nectar and honeydew sugars. However they exhibit differences in gustatory response and longevity, highlighting the potential for application of selective sugar sources in CBC.

The focus of ecology is thought to be changing from the traditional study of simple systems and interactions to approaches that consider the spatio-temporal variability of direct and indirect interactions among multiple trophic levels (Finke & Denno, 2002, 2004; Tscharrntke & Hawkins, 2002; Cardinale *et al.*, 2006). Studies focusing on individual processes in isolation such as single-species studies on biodiversity re-pest control could be too simple and underestimate levels of biodiversity required to maintain multifunctional ecosystems (Hector & Bagchi, 2007). This would take one step further the analysis of selective deployment of resource subsidies at the field level, and also increase understanding of the effect on herbivore populations. This would aid in more effective research and application of subsidies to other natural-enemy/ pest-crop systems. In order to use conservation biological control as part of an integrated pest management strategy, spatial scale, distribution and trophic webs must be thoroughly addressed case by case.

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