Use of selected flowering plants in greenhouses to enhance aphidophagous hoverfly populations (Diptera: Syrphidae)

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Abstract. The addition of floral resources in a crop is the most commonly used conservation biological control strategy. The influence of additional floral resources on the abundance of aphidophagous syrphids has been studied in Mediterranean sweet-pepper greenhouses, in southeast Spain. Sweet alyssum and coriander were the plant species used as flowering plants, distributed in the greenhouse in several monospecific patches. In our first experiment the influence on syrphid pre-imaginal stages (larvae and pupae) was studied and adult stages were studied in a second experiment. A higher number of pre-imaginal syrphids was recorded in two replicated greenhouses where flowers were introduced, compared with two control greenhouses (without additional floral resources). To evaluate the effect on adults, 4 greenhouses were divided into 2 plots in each greenhouse and flowers were introduced in one plot per greenhouse. More hoverfly adults were observed in the plots where flowers had been introduced than in the control. The three most abundant syrphid species found were *Eupeodes corollae*, *Episyrphus balteatus* and *Sphaerophoria rueppellii*. Specimens from these species were dissected, and their pollen content was analysed to assess the food potential of the introduced flowers. The three syrphid species fed on pollen from both the flowering plants, as well as on sweet-pepper pollen. This conservation biological control strategy is an effective method to enhance native syrphid populations in Mediterranean sweet-pepper greenhouses.

Résumé. **Utilisation de plantes fleuries en serres pour augmenter la population de syrphes aphidophages (Diptera : Syrphidae).** L'addition des ressources florales à une culture est une des stratégies les plus communes utilisées dans le contrôle biologique. On a étudié l'influence de ressources florales complémentaires sur l'abondance de syrphes aphidophages en cultures méditerranéennes de poivron en serres au sud-est de l'Espagne. On a introduit des plants de *Lobularia maritima* et de *Coriandrum sativum* disposés en groupes monospécifiques dispersés dans la serre. Dans une première expérience on a étudié l'influence des fleurs sur l'abondance des syrphides (larves et pupae). Dans une deuxième expérience, on a étudié l'influence sur celle des adultes. Un plus grand nombre de syrphes immatures a été enregistré dans les deux serres avec fleurs, en comparaison des deux serres témoins (sans autre ressource florale). Pour évaluer l'effet sur l'abondance des adultes, quatre serres ont été divisées chacune en deux parcelles. Des groupes de fleurs ont été introduites dans une parcelle par serre. On a observés plus de syrphes aphidophages adultes dans les parcelles avec fleurs que dans les contrôles. Les trois espèces de syrphes les plus abondantes ont été *Eupeodes corollae*, *Episyrphus balteatus* et *Sphaerophoria rueppellii.* Des spécimens de ces trois espèces on été disséqués pour une analyse pollinique afin d'évaluer le potentiel alimentaire des fleurs introduites. Les trois espèces de syrphidés se sont nourries du pollen des deux plantes à fleurs introduites ainsi que du pollen de poivron. Cette stratégie de contrôle biologique est une méthode efficace pour augmenter les populations natives de syrphidés aphidophages dans les serres méditerranéennes de poivron.

Keywords: Biological control, coriander, Mediterranean greenhouses, natural enemy, sweet alyssum, syrphids.

In sweet pepper (*Capsicum anuum* L.) greenhouses in the southeast of Spain, pest chemical control has been replaced by a biological control-based IPM (Integrated Pest Management) system, which consists of releasing natural enemies of the main pests combined with a reduction of synthetic pesticides. Aphids are one of the main pests found in sweet pepper crops (Sánchez *et al*. 2007), and in the study area biological control is mainly achieved by using parasitoids. During the spring, climatic conditions are optimal for the targeted aphid species, and pest outbreaks occur. This fact frequently disrupts the IPM program, and means that new biological control strategies are required.

Conservation biological control is the practice of enhancing the efficiency of natural enemies by modifying the environment (Eilenberg *et al.* 2001). This strategy can provide additional ecosystem services, such as the maintenance of biodiversity or pollination (Fiedler *et al.* 2008). Based on this fact, the provision of floral resources within or adjacent to crops is a conservation biological control strategy that increases the abundance of beneficial insects (Frank 1999;

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Sutherland *et al.* 2001; Pontin *et al.* 2006). It has been demonstrated that flowering plants can enhance the growth, survival, development and reproduction of some predators and parasitoids (Landis *et al.* 2000; Berndt & Wratten 2005). Moreover, it has even been demonstrated that they improve pest control (White *et al.* 1995; Hickman & Wratten 1996; Fitzgerald & Solomon 2004). Nevertheless, all the previous studies have been undertaken using outdoor crops, and in this article we provide the first data about the effect of introducing floral resources into natural enemies of greenhouse crops.

Mediterranean greenhouses are semi-open structures (Lindquist & Short 2004) with side walls that can be opened or closed manually in order to improve ventilation. This ventilation management facilitates a high degree of interaction of insect populations with other crops (Sánchez & Lacasa 2006) and can lead to an increase of natural enemies, which come from the outside environment into the greenhouse (Gabarra *et al.* 2004; Pineda & Marcos-García 2008a). The low isolation conditions of Mediterranean greenhouses compared with glasshouses from northern Europe mean that conservation biological control strategies for outdoor crops can also be applied to Mediterranean greenhouses.

The larvae of some syrphid species (Diptera: Syrphidae) are aphid predators and they have an important role as biological control agents, a fact that has been demonstrated in several studies (Chambers & Adams 1986, Rojo *et al.* 1996). Adults feed on pollen and nectar, and different syrphid species use floral resources selectively (Gilbert 1981). Nectar provides energy, while pollen provides the protein required for sexual maturing. This is particularly important in females for maturing successive batches of eggs (Schneider 1948). Flowering plants provide food resources, but also alternative prey, refuge against pesticides, or a habitat for sex meeting (Colley & Luna 2000). By analysing the gut content of foraging hoverflies it is possible to understand the function of a given flowering plant (Wratten *et al.* 2003).

Several flowering plants have been evaluated for hoverfly attraction, and the most visited species are sweet alyssum, *Lobularia maritima* L. Desv., coriander, *Coriandrum sativum*, L., buckwheat, *Fagopyrum esculentum* Moench, and phacelia, *Phacelia tanacetifolia* Benth. (Colley & Luna 2000, Ambrosino *et al.* 2006). Some characteristics of the candidate plant species should be considered prior to its introduction into a crop, such as its agronomic and economic compatibility with the crop, its relative attractiveness to natural enemies, but also its potential to attract pests and pathogens of the crop, and any environmental risk. In this work, two of these flowering plant species (sweet alyssum and coriander) have been used for the first time in sweet pepper crops and under the extreme climatic conditions that exist in Mediterranean greenhouses.

The objectives of the current study are to evaluate in sweet pepper greenhouses 1) the effect that selected flowering plants have on the abundance of pre-imaginal hoverflies preying on aphids in the crop; 2) the relative attractiveness of these flowering plants to adult aphidophagous syrphids; 3) whether the attraction of the introduced plants to syrphids is related to the performance of their flowers as a food resource.

Materials and methods

Study site

The study was conducted in commercial greenhouses of sweet pepper owned by the agricultural cooperative SURINVER S.C. located in the agricultural zone of Campo de Cartagena (southeast of Spain) over a three-year period (2004–2006). In this area sweet pepper is planted in December-January and it is grown until July-August. Other greenhouses and outdoor crops compose the surrounding landscape. Areas with natural vegetation have been removed, field margins included. All the greenhouses were of the "Almería" type, with a wooden or aluminum structure covered by thermal polyethylene. During the experimental period both the side walls (screen walls) and the zenithal windows of the greenhouses were completely open during the day. The greenhouses were managed under an IPM system. *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) releases against aphids took place during the experimental period once a week. On some occasions during the sampling years, pesticides such as pirimicarb, pyrethrum and soaps were applied, but never during the experimental periods.

Coriander and sweet alyssum seeds were sown in ten greenhouses by hand in January, at an approximate sowing rate of 18 gr of seed/Ha and 9 gr/Ha respectively. They were sown in alternate monospecific patches of five square metres each inside the greenhouse. Both plant species started producing flowers in March, but the coriander had its flowering peak in May whereas for sweet alyssum it was in June. Greenhouses were prepared with the selected flowering plants during the three years (2004–2006) of the study.

Records of immature stages

In 2004, 20 greenhouses were selected, and coriander and sweet alyssum were sown in ten of them as previously described. Due to the commercial character of the greenhouses, unexpected events occurred frequently and a higher number of study fields had to be prepared. Finally, four greenhouses were selected, from which two replicates were used as a control and two replicates were given additional floral resources. The aphid infestation was established with a severity scale (four levels), related to the status of the majority of the plants in the greenhouse. Level 1 meant aphids were present, but only on the terminal apex; level 2 corresponded to aphid presence on two parts of the plant, but the plant as a whole was not infested; level 3 meant that aphids had infested the whole plant, but without a clear reduction in growth; while level 4 showed a clear decrease in growth and defoliation. The four greenhouses had aphid population foci of *Myzus persicae* Sulzer (Hemiptera: Aphididae) (identified by a specialist) of a level 3 severity, and such severity did not change during the experimental period. Collection of immature syrphids took place between 5 May 2004 and 3 June 2004. This period corresponds with the period when both aphid and syrphid population levels are highest (Pineda & Marcos-García 2008a). The counts were taken in a randomised way around all of the aphid focus, by observation of 200 pepper plant leaves (sampling unit). All the leaves with eggs, larvae, or pupae of syrphids were collected in rearing cages (21 × 15 × 9 cm), as well as those leaves with such a high number of aphids that observation *in situ* was impossible. Syrphids were recorded and identified when possible in the laboratory. After this, the collected material was maintained in a climatic room (25 °C, 60-70% relative humidity) until the emergence of the syrphid adults, for confirmation of identification to species level by the authors.

Records of adult syrphids

During 2005 a study was carried out between 17 May 2005 and 24 May 2005, to assess the effect of the introduction of flower resources on the abundance of syrphid adults in the greenhouse. These days were during the period when the syrphid community had reached its population peak (Pineda & Marcos-García 2008a). In order to avoid the effect that aphids can have on syrphid attractiveness, the greenhouses were expected to be free of aphids during the experiment. Natural pyrethrum was ready to be applied in case of aphid presence, but aphids did not occur and the pesticide was not required. Four greenhouses (replicates) were selected, and they were divided with an anti-thrips nylon net in two plots of equal surface area (ca. 500m^2). In a preliminary experiment, adult syrphids were marked with different colours and released in each plot. No movement between plots and greenhouses was observed, and the conclusion was reached that the divisions were independent. Coriander and sweet alyssum were sown in one of the two plots from each greenhouse. Census walks were taken during 15 minutes (enough time to record the whole plot), and were repeated twice a day – once in the morning (between 8.00 and 11.00) and in an afternoon sampling (between 13.00 and 16.00). The census began at a different plot on each consecutive sampling to minimize temporal sampling bias and consisted of walking between every two lines of pepper plants, and shaking them simultaneously. All specimens of the aphidophagous syrphid species observed were recorded.

Pollen content of the three main species

During 2004, 2005, and 2006, adult specimens of the three most abundant syrphid species in the area were collected with hand nets during their population peak (Pineda & Marcos-García 2008a) in greenhouses with the selected flowering plants. *Eupeodes corollae* (Fabricius) specimens (n=15) were collected in April, *Episyrphus balteatus* (De Geer) (n=17) in May, and *Sphaerophoria rueppellii* (Wiedemann) (n=19) in June. The specimens were placed individually on glass slides and sexed in the laboratory. The abdominal contents were dissected out with needles and spread over the slide, following the methodology of Pérez-Bañón *et al.* (2003). The gut was opened, and pollen grains were mixed with a drop of distilled water. Then a piece of approximately 5 × 5 × 5 mm of glycerine jelly with fuchsin was placed on the slide, melted, and mixed with the pollen. A coverslip was applied and the preparation was observed with an optic microscope x400. Between 2000 and 3000 pollen grains were counted, to obtain an accurate estimation of the percentage of pollen from the different species being studied. Reference slides of pollen from specimens of the species of flowering plants introduced into the greenhouses and from sweet pepper were made as aids to identify pollen. Pollen grains from other plants species found in the gut were recorded as "others".

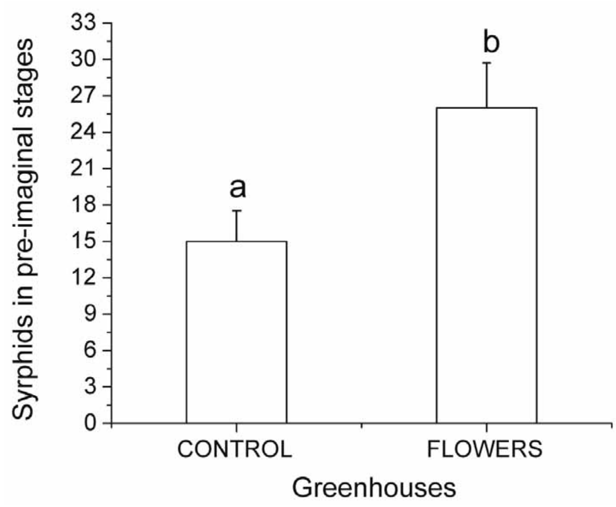

Figure 1
Mean number (±SE) of pre-imaginal hoverflies recorded in greenhouses with additional floral resources and control (n = 18 observations per treatment). Different letters indicate significant differences (Mann-Whitney test, p < 0.05).

Table 1. Mean (±SE) numbers of hoverfly larvae recorded per sample (200 leaves of sweet pepper) and greenhouse. P < 0.05 indicates significant differences between the two treatments (Mann-Whitney test).

Treatment	*Episyrphus balteatus*	*Eupeodes corollae*	*Sphaerophoria rueppellii*	*Sphaerophoria scripta*	*Scaeva albomaculata*
control	8.3 ± 7.1	4.1 ± 4.8	2.6 ± 4.2	0.0 ± 0.0	0.0 ± 0.0
with flowers	12.4 ± 9.2	5.4 ± 5.6	7.9 ± 9.9	0.4 ± 0.7	0.1 ± 0.2
z (df = 1)	1.4	1.1	2.4	2.4	1.0
p	0.17	0.27	0.02	0.02	0.32

Table 2. Percentage of pollen grains (%) found in gut contents of the three most abundant syrphid species. Different capital letters within a column indicate significant differences between syrphid species (Dunn's test; p < 0.05). Lower-case letters within a row indicate significant differences between pollen types (modification of Dunn's test; p < 0.05).

Syrphid species	*L. maritima*	*C. anuum*	*C. sativum*	"Others"	n	χ^2 (df = 3)	p
E. corollae	6.8 aA	38.9 bA	7.2 aA	47.2 bA	15	22.9	0.000
E. balteatus	5.8 aA	24.4 aA	52.7 aB	17.2 bB	17	7.9	0.049
S. rueppellii	51.2 aB	30.2 aA	2.1 bA	16.5 aAB	19	18.1	0.000
χ^2 (df = 2)	16.5	1.4	17.9	8.1			
p	0.000	0.50	0.000	0.02			

Statistical analysis

Data did not show a normal distribution, therefore non-parametric tests were used to analyse them. The effect of flowering plants introduction on pre-imaginal syrphid counts was analysed using the Mann-Whitney test. Data represent the mean number of syrphids (±SE) per sample of 200 leaves per greenhouse. To evaluate the effect on adult syrphids, the Friedman test for related samples was used. In this case data represent the mean number of syrphids recorded in 15 min per plot.

A Friedman test for related samples was used to compare differences between pollen types, followed by a modified Dunn's method for pairwise multiple comparisons (Gardiner 1997). To compare differences between syrphid species, a Kruskal-Wallis test for independent samples was applied, followed by the Dunn method for pairwise multiple comparisons. Data were analysed with the SPSS statistical package (SPSS 2004), and the non-parametric multiple comparisons were performed manually.

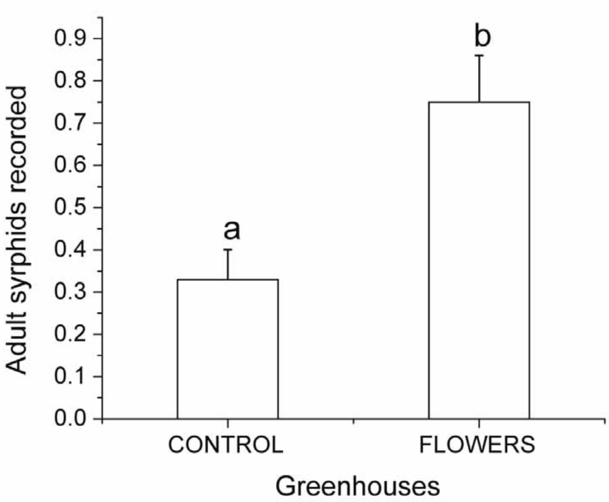

Figure 2
Mean number (±SE) of adult hoverflies recorded in plots with additional floral resources and control (n = 64 observations per treatment). Different letters indicate significant differences (Friedman test, p < 0.05).

Results

Effect on immature stages

Five hoverfly species were found feeding on the aphid pest *Myzus persicae*, from a total of 742 immature syrphids collected: *Episyrphus balteatus* (50%), *Sphaerophoria rueppellii* (26%), *Eupeodes corollae* (23%), *Sphaerophoria scripta* (L.) (0.9%) and *Scaeva albomaculata* (Macquart) (0.1%). The number of Syrphidae (fig. 1) found in greenhouses with flowering plants was significantly higher than in the controls (z = 2.2, df = 1, p = 0.028). When a mean comparison by species was carried out (tab. 1), each Syrphidae species was more abundant in greenhouses with flowering plants than in the control.

Effect on adult hoverflies

Four aphidophagous syrphid species could be identified *in situ*, by adult census during an eight-day sampling period in 2005: *S. rueppellii* (71%), *S. scripta* (11%), *E. corollae* (6%) and *S. albomaculata* (1%). We also observed several specimens of the genus *Paragus* (11%), but they could not be identified at species level during the census walks. The introduction of flowering plants in the greenhouses had a significant positive effect on adult hoverfly numbers (χ^2 = 6.4, df = 1, p = 0.01) (fig. 2).

Pollen content of the three main species

Pollen from the introduced flowering plants and from sweet pepper is used as a food resource by the three syrphid species studied, as was observed in the gut contents (tab. 2). *Eupeodes corollae* consumed more pollen from sweet pepper and "others" than from the introduced flowering plants. For *E. balteatus*, there were no differences in the consumption of pollen types from the introduced plants and sweet pepper. *Sphaerophoria rueppellii* consumed significantly less pollen from coriander than from the other studied plants. Regarding the different pollen consumption by syrphid species, sweet pepper pollen was the only type that was found in similar proportions in the three syrphid species.

Discussion

The results of the current study confirm that the provision of extra floral resources in sweet-pepper greenhouses leads to an increase of aphidophagous hoverflies in the greenhouses. This fact has been demonstrated for adult and pre-imaginal stages, which control pests. Nevertheless, at the end of the experiment with pre-imaginal stages, the economical threshold was exceeded and growers applied pesticides against aphids in all the greenhouses. Nowadays biological control agents, syrphids included, are highly available and the increased abundance of syrphids observed with this strategy shows a direct economical benefit from not purchasing the equivalent amount of natural enemies. Although they have not been studied in this work, other natural enemies such as anthocorids or parasitoids are also attracted by flowering plants (Fitzgerald & Solomon 2004), and this fact increases the economical potential of this strategy.

Several studies have observed a higher number of hoverflies when introducing flowering plants in an outdoor crop (Hickman & Wratten 1996; Fitzgerald & Solomon 2004; Pontin *et al.* 2006), but this is the first time that this has been proven for commercial greenhouses. The special character of Mediterranean greenhouses makes this possible, as their semi-open structure leads to the natural presence of natural enemies (Gabarra *et al.* 2004; Pineda & Marcos-García 2008a). The most abundant species found in the immature stages study are the three most abundant aphidophagous hoverflies in the region (Pineda & Marcos-García 2008a), *E. balteatus*, *E. corollae* and *S. rueppellii*. Our results suggest that this manipulation strategy of introducing floral resources is valid for each of them. In previous works we assessed other strategies to enhance native syrphid populations in greenhouses, with cultural methods such as the opening of greenhouse side walls (Pineda & Marcos-García 2008a), and conservation biological control strategies, such as the provision of aphid reservoirs (Pineda & Marcos-García 2008b) being effective. A combination of the three strategies could be a promising way of increasing the effectiveness of native syrphids in greenhouses.

Syrphids lay higher numbers of eggs at higher densities of prey, with aphids being the main stimulus for oviposition (Chandler 1968). In a preliminary bioassay, adult abundance was tested with aphid presence and therefore the effect of the flowers was disguised. To evaluate the effect of flowers on adult syrphids with aphid presence, it would be necessary to work with the same aphid density, and for that the introduction of aphids and their monitoring is required. Sweet pepper has a high crop value/unit area, and controlled aphid introduction in several replications is unrealistic in commercial greenhouses. Consequently, the adult syrphid study was performed without aphids, to assess the effect of introducing flowering plants only, which had a significant effect on adult abundance.

Sweet alyssum and coriander were introduced in greenhouses over a period of three years (2004-2006), and growers had no objections to this, since disadvantages of their use, such as the attraction of pests or visible growth reduction on the neighbouring sweet-pepper plants were not observed. Both species could be colonised by the aphids *Myzus persicae* and *Macrosiphum euphorbiae* (Thomas), but only when the aphid infestation of the crop was extremely high, and no other pest species were observed on them. Nevertheless, other risks need to be evaluated further, such as the potential of these flowering plants to act as aphid reservoirs (Morales *in lit.).*

With the analysis of pollen content, we confirm the hypothesis that syrphid attraction by introducing flowers into greenhouses is related to feeding behaviour of the adult syrphids. The proportion in which pollen is present in the syrphid gut, is different for each syrphid species, and this is related to syrphid and flower plant phenology. Both plant species start producing flowers in March, but coriander had its flowering peak in May whereas for sweet alyssum it was in June. Pineda & Marcos-García (2008a) found that the three most abundant syrphid species in the greenhouses show a temporal succession: *E. corollae* has its population peak in April, *E. balteatus* in May, and *S. rueppellii* in June. According to this fact, *E. corollae* ingested a small proportion of coriander and sweet alyssum pollen, and in contrast a high proportion of sweet-pepper and “others” pollen was found. The introduced flower pollen was not the main item ingested by *E. corollae,* because when adult captures took place, these flowers were present in the greenhouses but it was not their maximum flowering period. *Episyrphus balteatus* captures took place in May, and coriander was the most numerous pollen found in their guts, coinciding with its flowering peak. Similarly, *S. rueppellii* was captured in June, and the pollen type most ingested was from sweet alyssum, the plant with the last flowering peak.

The sweet-pepper pollen was the only one whose proportion in syrphid guts was similar for the three syrphid species, probably because from the four pollen types studied, sweet pepper is the only one whose production is constant during the spring. The fact that sweet pepper consumption is independent of the presence of the additional floral resources is proof that this conservation biological control strategy does not influence crop pollination negatively, while it attracts syrphids to the greenhouse. Colley & Luna (2000) suggested that to enhance the effectiveness of this strategy, it is recommended to introduce several plants with overlapping flowering periods. With the combination presented here it is possible to obtain a longer flowering period than if just one species were introduced, and synchronised with the moment when syrphids are most abundant. Further screening on other plant species is required, to obtain a longer flowering period and under the high temperatures that exist in Mediterranean greenhouses.

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