

Olfactory attractiveness of flowering plants to the parasitoid *Microplitis mediator*: potential implications for biological control

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Abstract In agricultural landscapes, the lack of floral nectar can be a major difficulty for nectar feeding parasitoids. This problem can be reduced by the addition of suitable wildflowers. To date, flowers have mainly been studied in terms of effects on parasitoid fitness, not taking into account the essential role of flower attractiveness for foraging parasitoids. This study experimentally tested the olfactory attractiveness of five wildflowers (bishop's weed, cornflower, buckwheat, candytuft, and oregano) to the parasitoid *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae). We conducted choice experiments in a Y-tube olfactometer to test the attractiveness of flowers against air, and relative attractiveness in paired choice tests. Our results showed that all the flowers were highly attractive and that in paired choice tests cornflower and candytuft were equally attractive and

more attractive than buckwheat. These results indicate that *M. mediator* has evolved innate preferences that could be effectively exploited in biological control.

Keywords Hymenoptera, Braconidae · Parasitic wasp · Attraction · Flower odor · Floral

Introduction

Many agricultural landscapes offer unfavourable conditions to natural enemies such as arthropod predators and parasitoids. Frequent and intense disturbances, e.g. pesticide applications or harvest, can influence insect diversity and—in the case of parasitoids—reduce species richness, abundance and effectiveness (Landis and Menalled 1998; Naranjo and Ellsworth 2009). The scarcity or absence of flowers in crop fields can also be a critical issue for the survival of natural enemies, because floral nectar is a major food source for many of them (Hogg et al. 2011; Letourneau and Altieri 1999). These unfavourable conditions are accentuated in annual monocultures which represent temporary habitats that are less stable, and thus exposed to higher levels of disturbance, than perennial cultures (Ferro and McNeil 1998; Landis et al. 2000). This can result in lower establishment rates of natural enemies and consequently lower success in controlling the pests (Landis et al. 2000).

Conservation biological control can mitigate these problems through habitat management, i.e. by

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modifying the habitat to enhance the abundance and activity of natural enemies and consequently improve the control of pest populations (Jonsson et al. 2008; Landis et al. 2000; Naranjo and Ellsworth 2009). For example, wildflower strips (i.e. wildflowers planted along the field margin) or companion plants (i.e. non-crop plants inserted within the field and alternating with the crop plants) can be added as alternative food sources (Lee and Heimpel 2005; Pfiffner et al. 2009; Ponti et al. 2007). Like other intercrops and ground covers, they also represent shelter habitats that offer protection to the natural enemies during pesticide applications, harvest, overwintering or in case of unfavourable weather (Griffiths et al. 2008; Landis et al. 2000; Pfiffner and Luka 2000).

Wildflower strips and companion plants have already been used in the field to enhance predators and parasitoids, but the results are mixed. Some studies show that the provision of floral resources increases parasitism rates (Ellis et al. 2005; Lavandero et al. 2005; Ponti et al. 2007), while others show no effect (Berndt et al. 2002), negative effects (Bone et al. 2009) or effects that vary depending on the year (Lee and Heimpel 2005) or the field location (Pfiffner et al. 2009). This inconsistent pattern of effects of wildflower strips and companion plants stresses the importance of studying in detail the decision making and preferences of predators and parasitoids for food sources. In particular, conducting flower screening to identify suitable flowering plants that will effectively (1) attract natural enemies into the field and closer to the pest, and (2) offer the natural enemies accessible nectar as food resource to increase their lifespan and/or the fecundity (Wäckers 2004) is important to improve the effects of wildflowers on parasitoid performance.

In the case of parasitoids, flower screening has so far focused on the identification of wildflower species that enhance the survival and/or the fecundity of the studied species (Baggen and Gurr 1998; Géneau et al. 2012; Lavandero et al. 2006; Nafziger and Fadamiro 2011; Winkler et al. 2006; Witting-Bissinger et al. 2008). But before being able to feed on flowers, foraging parasitoids have to locate them by using for example olfactory (Desouhant et al. 2005; Leius 1960; Patt et al. 1999; Siekmann et al. 2004; Takasu and Lewis 1996) and/or visual cues (Begum et al. 2004; Kugimiya et al. 2010). Food sources that are highly attractive are thus more likely to be visited than food sources that are poorly detectable (Wäckers 2004).

Bianchi and Wäckers (2008) used a spatially explicit simulation model to explore how the attractiveness and the nectar availability of flowering field margins affect parasitoid and ultimately pest populations. This model predicts that the attractiveness of flowers is an important aspect of parasitoid population dynamics that should be taken into account when selecting flowering plants used for habitat management. Despite its predicted importance for effective conservation biological control of pest species, the attractiveness of flowering plants to parasitoids remains poorly studied (Kugimiya et al. 2010; Leius 1960; Wäckers 2004).

The aim of this study was to investigate the olfactory attractiveness of five different flowering plants to females of the parasitoid *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae). *Microplitis mediator* is an important parasitoid of *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae) (Lauro et al. 2005), a major cabbage pest in Europe (Finch and Thompson 1992). It is also a generalist parasitoid that has been reported on about 40 different Noctuidae hosts (Mir Khan 1999) and has already been used for biological control (Li et al. 2006). Mass releases of *M. mediator* led to successful control of cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) populations in Northwestern China (Li et al. 2006). Furthermore the longevity and fecundity of *M. mediator* are greatly enhanced by the provision of suitable sugar (Luo et al. 2010) or nectar food sources (Géneau et al. 2012), but flower attractiveness to this parasitoid has not been investigated so far. To test the olfactory attractiveness of the flowering plants, we used choice experiments in a Y-tube olfactometer. We first tested whether the different wildflowers were attractive to female *M. mediator* by comparing the flower odours to two control odour sources (air and a piece of stem of the same plant), and then tested the flowers against each other in paired choice experiments to assess olfactory preferences of *M. mediator* among these flowers.

Materials and methods

Plants and parasitoids

We tested the attractiveness of bishop's weed, *Ammi majus* L. (Apiaceae); cornflower, *Centaurea cyanus* L. (Asteraceae); buckwheat, *Fagopyrum esculentum*

Moench (Polygonaceae); candytuft, *Iberis amara* L. (Brassicaceae); and oregano, *Origanum vulgare* L. (Lamiaceae). These flowering plants have properties that make them promising candidates for habitat management, e.g. beneficial effects on insects' longevity and/or fecundity (Géneau et al. 2012; Lavandero et al. 2006; Lee and Heimpel 2008; Nafziger and Fadamiro 2011; Winkler et al. 2006; Witting-Bissinger et al. 2008), attractiveness to some parasitoid species (other than *M. mediator*) (Wäckers 2004), the production of easily accessible extrafloral nectar (Koptur 2005; Winkler et al. 2009), or subsidiary uses like medicinal use (Ammon et al. 2006; Fabre et al. 2000) that could be of interest for farmers.

Flowering plants were grown from seeds in GroBanks (CLF Plant Climatics, Germany) at 21 ± 2 °C, 40 ± 10 % r.h. and 12L:12D photoperiod. Seeds were planted in trays in soil (Einheitserde Classic, Gebrüder Patzer GmbH & Co.KG, Germany) fertilized with 3 g l^{-1} of Tardit 3 M (Hauert HBG Dünger AG, Switzerland) and transplanted into pots (12 cm diameter, 10 cm height) after three to four weeks with the same amount of fertilizer. Plants were checked on a daily basis and watered as needed until they bloomed.

Microplitis mediator pupae came from a laboratory population initiated by individuals collected on *M. brassicae* in Brussels sprout fields near Wageningen (the Netherlands) and reared at Wageningen University. The parasitoid rearing was then established in a climate chamber at 23 ± 1 °C, 60 ± 10 % r.h. and 16L:8D photoperiod. Parasitoids were reared on *M. brassicae* larvae fed on white cabbage *Brassica oleracea* L. var. *capitata* L. (Brassicaceae). Adults of *M. mediator* hatched in the room where the experiments took place, at 16L:8D photoperiod. All the females used for the experiments were food deprived (i.e. provided with only water from emergence to the behavioural test), naïve (i.e. no contact with the tested odour sources previous to the experiments) and between 24 and 72 h old. Females were used only once and discarded after the behavioural test.

Experimental setups

Y-tube olfactometer

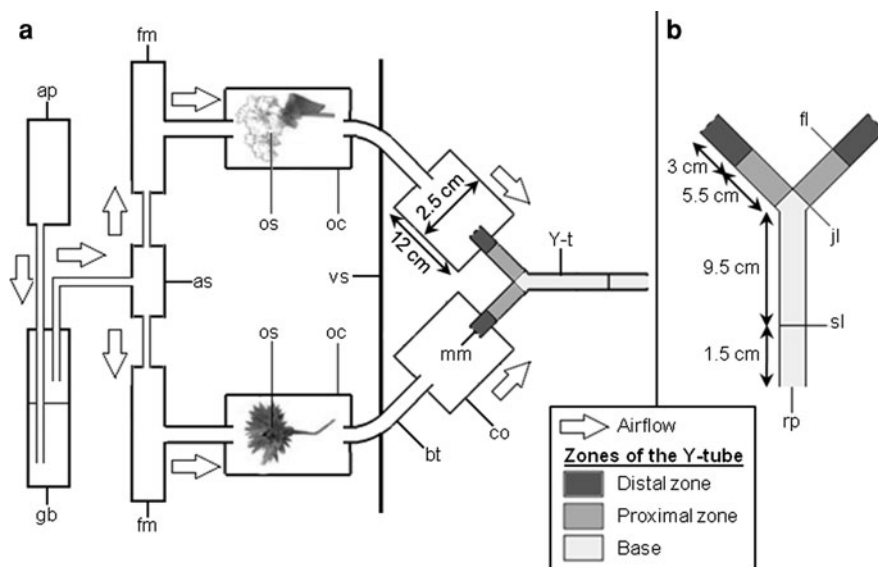
The attractiveness of the different flowers was assessed over three experiments where the olfactory

preference of the parasitoid was tested in olfactory choice tests thanks to a Y-tube olfactometer (Fig. 1). The Y-tubes had an internal diameter of 1 cm, and the two arms of the Y-tube were connected through a connector to a bent tube, which in turn was connected to a container containing an odour source (Fig. 1a). As odour sources, one flower (for *C. cyanus*) or one inflorescence (for *A. majus*, *F. esculentum*, *I. amara* and *O. vulgare*) was cut and the stem was wrapped in a wet piece of cotton to prevent wilting. The flowers were used within one hour and a half after cutting. The inflorescences of *A. majus* were sometimes too big for the container, so 5–8 flowers of the same inflorescence were cut and put together like a small bouquet in the container. To ensure the right functioning of the setup, we used honey (Waldhonig, Migros, Switzerland) as a positive control odour source, as preliminary experiments had shown that it was attractive to female *M. mediator*. To prepare the honey odour source, one to two drops of honey were spread on a piece of filter paper (Whatman, GE Healthcare, United Kingdom) previously humidified with water so that the honey covered approximately 2 cm^2 . A white cardboard shield was placed between the Y-tube and the odour source containers to prevent the insects from having any visual information about the odour sources and thus avoid potential confounding effects due to visual attractiveness of the flowers. A metal mesh at the end of each arm prevented the insects from entering the containers and contacting the odour sources.

For behavioural scoring, the Y-tube was divided by thin lines into different zones which represented the proximity to the odour (Fig. 1b). The zone between the opening of the central tube and the junction lines (8.5 cm before the end of each arm) was referred to as the “base” of the Y-tube. Each arm was divided into the “proximal zone” between the junction line and the finish line (3 cm before the end of the arm), and the “distal zone” between the finish line and the metal mesh at the end of the arm.

Experiments took place in a dark room to avoid directional light, and a light bulb (375 lux) was placed centrally approximately 50 cm above the setup. Air was pushed through the olfactometer by a vacuum pump, filtered on glass cotton and activated charcoal to limit impurities in ambient air, humidified in a gas washing bottle containing demineralised water, and led into each arm of the Y-tube at 0.433 l min^{-1} , so that the airflow at the release point of the insects was

Fig. 1 Experimental setup. Top view of **a** the setup and **b** the Y-tube. Two odour sources are placed in the odour source containers. A parasitoid is released in the Y-tube at the release point and has to make a choice between the two odours at the junction line. ap air pump, as air splitter, bt bent tube, gb gas washing bottle, co connector, fl finish line, fm flow meter, jl junction line, mm metal mesh, oc odour source container, os odour source, rp release point sl start line, vs visual shield, Y-t Y-tube



0.866 l min^{-1} . Flow meters (Teflonrotameter 150 mm, Analyt-MTC, Germany) were used to control the airflow.

The Y-tubes, connectors and bent tubes were made of glass, and all the other tubes used to connect parts of the setup or transfer the insects in the Y-tube were made of Teflon (PTFE) to allow the cleaning with solvents. The cleaning was done by rinsing the parts with hexane (Roth AG, Switzerland), acetone (Roth AG, Switzerland) and dichloromethane (Roth AG, Switzerland) (once each, in this order). Y-tubes were cleaned at the end of each day (i.e. after one to five replicates). On a given day, one Y-tube was used per odour source, and odour sources were always connected to the same arm of the Y-tube to prevent odours from mixing with each other. Every container was connected to the same bent tube and connector during the entire duration of an experiment and all these parts were cleaned after each experiment.

Behavioural tests

To start a measurement, one female parasitoid was transferred from the cage to the Y-tube in a 2.5 cm Teflon tube closed on one end by a metal mesh. The tube was fixed to the base of the Y-tube, and the test began when the female crossed the start line (9.5 cm before the bifurcation). The time the female spent in each zone was recorded in real time during 5 min using JWatcher V1.0 (Blumstein and Daniel 2007).

The small fraction of females that did not cross the start line within five minutes were considered as non-responders and removed from the experiment.

In experiment 1, we tested the absolute attractiveness of the flowers and of honey to female *M. mediator*, i.e. whether the odour sources were attractive. An odour source was considered attractive when the parasitoids exhibited a preference for it. We considered two measurements of female preference. The first choice on one hand was defined as the arm in which females crossed the junction line for the first time, to test whether a preference could be detected as soon as the females entered an arm or whether the females needed some exploration before displaying a preference. The residence time on the other hand was obtained by the time females spent in the distal zones of the two arms. Females were considered to have a lasting preference for an odour source when they spent significantly more time in the distal zone of its arm than in the distal zone of the other arm. The flowers tested in experiment 1 were *A. majus*, *C. cyanus*, *F. esculentum*, *I. amara*, and *O. vulgare*. The odour sources were compared to a piece of filter paper humidified with tap water as a negative control. The filter paper was used to add humidity in the arm to balance the extra humidity produced by the flower. In a control experiment, the humid filter paper did not significantly affect the preferences of the female parasitoids in the olfactometer compared to air (Wilcoxon signed-rank test, $n = 50$, $V = 691.5$,

$p = 0.099$) and, hence, was considered a neutral control.

In experiment 2, we tested whether the results obtained in experiment 1 reflected a specific response to the volatiles from the flowers and were not just due to volatiles emitted by the cut stem. The experiment was set up exactly like experiment 1, except that a flower or inflorescence was compared to a piece of stem of the same flowering plant. The piece of stem was taken directly under the flower or inflorescence used as an odour source and was approximately the same length as the stem left under the flower or inflorescence, so that the flower or inflorescence was the only difference between the two arms. For time and practical reasons, we left aside *A. majus* and *O. vulgare*, which are difficult to rear, limiting their usefulness for application in the field, and focused on *C. cyanus*, *F. esculentum*, and *I. amara*.

In experiment 3, we tested the relative attractiveness of the different odour sources to females *M. mediator* using paired-choice tests in order to assess which floral scent was most attractive. A flower was considered more attractive than another when the parasitoids exhibited a preference for it, and the first choice and residence time were measured like in the previous experiments. The setup was like in experiment 2, except that odour sources were compared against each other in pairs.

All the odour sources (experiments 1 and 2) or pairs of odour sources (experiment 3) were tested in a randomized order for each replicate, and one individual female was used for each odour source or pair of odour sources. Odour sources were renewed for each replicate and their positions (i.e., left or right in the Y-tube) alternated between replicates to randomize possible side-effects on the preferences of *M. mediator*. One to five replicates were performed on a given experimental day.

Statistics

Statistical analyses were conducted using R 2.13.2 (R Development Core Team 2011). We treated as non-responders the females that never entered any distal zone for the analysis of the residence time, and those that never entered any proximal zone for the analysis of the first choice, and removed them from the analysis. For the residence time, the total time spent in the distal zone varied between females, so we

defined a preference index by calculating the ratio of the total time spent in the distal zone of one arm over the total time spent in the distal zones of both arms. The data were not normally distributed, even after transformation, so we performed a Wilcoxon signed-rank test to test whether there was a significant preference for one arm or the other (i.e. whether the preference index was significantly different from 0.5). For experiments 1 and 2, we used a generalized linear model with quasibinomial data distribution to test for significant differences among all the odour sources. We performed two Pearson's χ^2 tests for the analysis of the first choice, first to test whether the numbers of females that first chose one arm or the other were significantly different, and then, for experiments 1 and 2, to test whether there was a significant difference in the first choice among the odour sources.

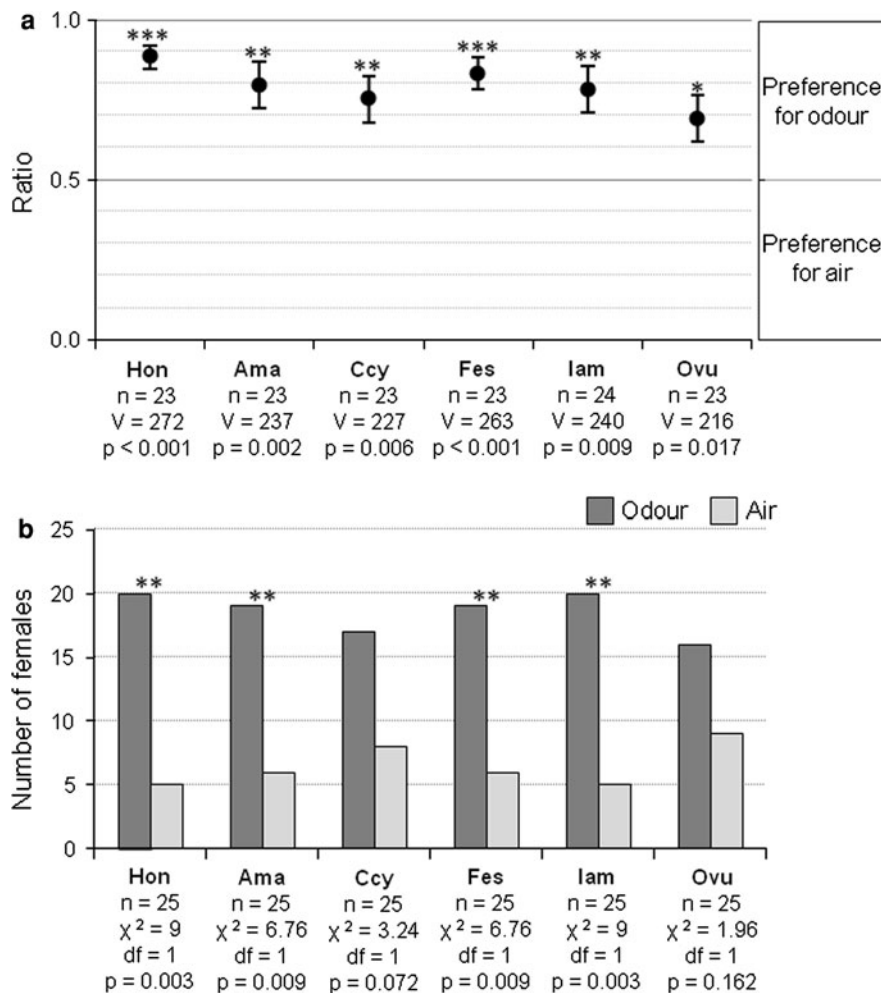
Results

In experiment 1 where we tested the absolute attractiveness of flowers compared to air as a neutral control, the parasitoids spent significantly more time in the arm connected to the odour source over air for all the tested odour sources (flowers and honey) (Fig. 2a). There was no significant difference in the residence time between the odour sources ($\chi^2 = 10.092$, $df = 5$, $p = 0.073$). Except when offered *C. cyanus* and *O. vulgare*, significantly more parasitoids showed a first choice towards the odour over air (Fig. 2b), and there was no significant difference in the first choice among the odour sources ($\chi^2 = 2.807$, $df = 5$, $p = 0.730$).

In experiment 2, where we tested the attractiveness of flowers in comparison with the stem, the parasitoids spent significantly more time in the distal zone of the arm connected to the flowers over the stem for the three flowers tested (Fig. 3a). Like in experiment 1, there was no significant difference in the residence time between the flowers ($\chi^2 = 3.083$, $df = 2$, $p = 0.214$). Except when offered *F. esculentum*, significantly more parasitoids showed a first choice for the flower over the stem (Fig. 3b), and there was no significant difference in the first choice among the three flowers ($\chi^2 = 4.656$, $df = 2$, $p = 0.098$).

In experiment 3 where we tested the relative attractiveness of *C. cyanus*, *I. amara* and *F. esculentum* in paired-choice tests, distinct differences in olfactory attractiveness emerged between flowers.

Fig. 2 **a** A Mean (\pm SE) ratio of the total time spent in the distal zone of the odour arm over the total time spent in the distal zones of both arms (odour and air). A ratio of 0.5 indicates no preference, whereas a ratio significantly superior or inferior to 0.5 indicates a preference for the odour or the air (i.e. a repellence of the odour), respectively. Asterisks indicate a significant deviation from 0.5 (Wilcoxon signed rank test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **b** Number of females that first chose the odour arm (dark grey) or the air arm (light grey). Asterisks indicate significant differences (χ^2 test, * $p < 0.05$, ** $p < 0.01$). *Ama* *Ammi majus*, *Ccy* *Centaurea cyanus*, *Fes* *Fagopyrum esculentum*; *Hon* Honey, *Iam* *Iberis amara*, *Ovu* *Origanum vulgare*



Parasitoids significantly preferred *C. cyanus* and *I. amara* over *F. esculentum*, whereas they showed no significant preference between *C. cyanus* and *I. amara* (Fig. 4a). This pattern was mirrored in terms of the first choice (Fig. 4b).

Discussion

The aim of this study was to investigate the olfactory attractiveness of five different flowering plants to female *M. mediator* to assess their suitability for conservation biological control in cabbage fields. The flower species we tested were all highly attractive when compared to air, both in terms of first choices and residence time, implying strong innate preferences by naive female *M. mediator*. This result is in line with a previous study which showed that naive females of

the parasitoids *Pimpla turionellae* (Linnaeus) (Hymenoptera: Ichneumonidae), *Heterospilus prosopidis* (Viereck) (Hymenoptera: Braconidae) and *Cotesia glomerata* (Linnaeus) (Hymenoptera: Braconidae) exhibited innate preferences for flower odours tested against air (Wäckers 2004). In our case, the attraction was maintained when *C. cyanus*, *F. esculentum* and *I. amara* flowers or inflorescences were compared to a piece of stem of the same plant, which demonstrates that female *M. mediator* were really attracted by the flower odours and not by volatiles emitted by other parts of the plant such as the stem. Kugimiya et al. (2010) showed a similar specific response to odours of mustard flowers *Brassica rapa* L. (Brassicaceae) in the parasitoid *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae). The results of experiment 3 showed that although all the flowers were similarly attractive when compared to air, some of them were more attractive

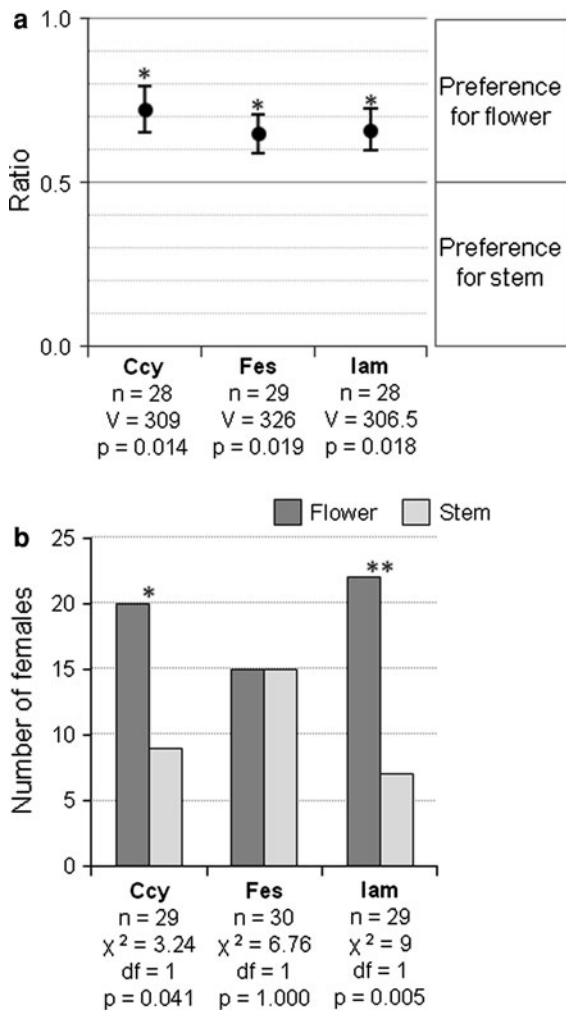


Fig. 3 a Mean (\pm SE) ratio of the total time spent in the distal zone of the flower arm over the total time spent in the distal zones of both arms (flower and stem). b Number of females that first chose the flower arm (dark grey) or the stem arm (light grey). See Fig. 2 for abbreviations and plot details

than others when tested by pairs. *Centaurea cyanus* and *I. amara* were equally attractive when tested in paired choice, and they were both more attractive than *F. esculentum* which was the least attractive flower. This highlights the fact that absolute attractiveness is certainly important to identify flowers that are neither neutral nor repulsive to the parasitoids. But how attractive a flower is in the end and, hence, how easily it may be located by a foraging parasitoid, also depends on other available flowers.

The low attractiveness of *F. esculentum* compared to the two other plants can be explained by different mechanisms that remain to be elucidated in further

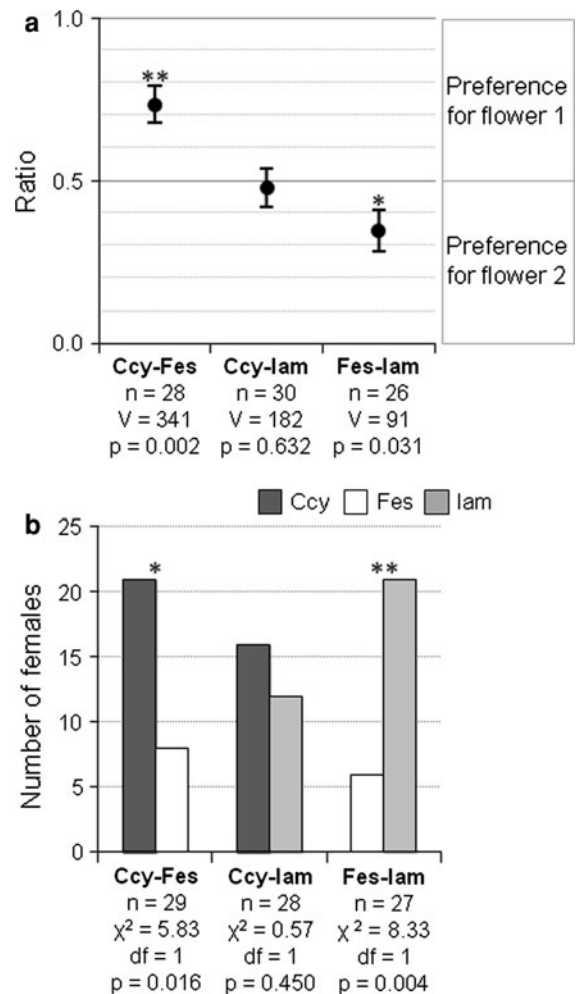


Fig. 4 a Mean (\pm SE) ratio of the total time spent in the distal zone of one of the flower arm over the total time spent in the distal zones of both flower arms. b Number of females that first chose the *Centaurea cyanus* (Ccy, dark grey) arm, the *Fagopyrum esculentum* (Fes, white) arm, and the *Iberis amara* (lam, light grey) arm. Pair names give the two flowers tested against each other separated by a dash. Flowers 1 and 2 refer to the first and second flower in the pair name, respectively. See Fig. 2 for plot details

experiments. The results of experiments 1 and 2 show that *F. esculentum* inflorescences produce attractive volatile molecules. A first hypothesis is that the molecules produced by *F. esculentum* are qualitatively less attractive or detectable to *M. mediator* than those produced by *C. cyanus* and *I. amara*. Second, the molecules produced by the three plants may be qualitatively equally attractive or detectable, but flowers of *F. esculentum* may produce them in lower quantities. Evidence supporting the importance of

volatile quantity on parasitoid attraction was provided in a study about *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) (Turlings et al. 2004). Females of this parasitoid in fact respond in a dose-related manner to host induced green leaf volatiles and are more attracted in a six-arms olfactometer to the arm connected to the highest number of plants (Turlings et al. 2004) If females *M. mediator* exhibited the same kind of dose-related response to find their food, they would then be more attracted by the flower emitting the highest amounts of chemical attractants when given the choice between two flowers producing equally attractive volatiles. In the current setup, we could not distinguish if *F. esculentum* inflorescences were less attractive than the flowers of *C. cyanus* and the inflorescences of *I. amara* because they emitted volatiles that were qualitatively less attractive or because they emitted quantitatively less volatiles. Further tests are needed to collect, identify and quantify the volatiles emitted by the different flowering plants and to test whether female *M. mediator* respond in a dose related manner. It has to be noted that cutting the flowers could also have an influence, if one flower species were more stressed by the cutting than others. Although we cannot completely exclude this hypothesis, it seems unlikely since we used the flowers shortly after cutting, as has previously been done in another study investigating flower attractiveness to parasitoids (Wäckers 2004). The analysis of flower volatiles has also often been done on cut flowers or inflorescences (Borg-Karolson et al. 1993; Raguso et al. 2006; Verdonk et al. 2005), or even on separate flower parts (Flamini et al. 2003), and it has been shown in *Lysimachia* species that cutting did not influence floral scent emission immediately after cutting (Schäffler et al. 2012).

Our results have implications for biological control, as we have shown that five different flowering plants that could be or are already used in wildflower strips or as companion plants are also attractive to *M. mediator* when offered as the sole wildflower (i.e. none of them is neutral or repulsive). However, some of them seem to be better suited than others. The attractiveness of the flowering plants did not correlate fully with parasitoids' performance on these flowers, a finding that is also in line with previous results on other parasitoid species (Wäckers 2004). In a parallel study, Géneau et al. (2012) found that *C. cyanus* and *F. esculentum* greatly enhanced the longevity and fecundity of *M.*

mediator compared to water, whereas *I. amara* had no detectable beneficial effect. Our expectation for adaptive olfactory attractiveness of plants that enhance parasitoid fitness was confirmed for *C. cyanus*. However, we did not expect *I. amara* to be attractive, let alone more attractive than *F. esculentum*. The lack of correlation between attractiveness and benefit to the parasitoids for these two flowering plants is hard to explain at first sight. It could result from the fact that in the field parasitoids are likely to encounter a broad range of different flowers, depending on the place where they emerge, so it would not be advantageous to develop a specific attraction to one or a few floral scents (Wäckers 2004). Conversely, to maximize the chances of finding flowers (and thus food) in an unknown environment, they may respond to floral volatile compounds that many different flowering plants have in common, even at the risk that some of these plants do not provide suitable nectar. Parasitoids should then refine their ability to recognize suitable flowers through learning, an ability that was shown in females of the related species *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), which are able to associate odours with the presence of food and became more and more accurate in their choice with increasing number of odour experiences (Takasu and Lewis 1996). It is likely that *M. mediator* have evolved similar mechanisms of associative learning.

Flower attractiveness could of course be affected by other factors like visual cues (Begum et al. 2004; Kugimiya et al. 2010). But given the innate preferences of female *M. mediator* for the different flower odours shown in our study, it seems that this parasitoid evolved mechanisms to respond to distinct plant volatiles and use these olfactory cues to locate available food sources efficiently. Based on that, our results provide a panel of interesting flowers attractive to *M. mediator* that could be tested for their attractiveness and beneficial effects in the field. *Centaurea cyanus* appears to be the most promising one, because of its high absolute and relative attractiveness to the parasitoid and beneficial effect on its longevity and fecundity (Géneau et al. 2012). Although *I. amara* was as attractive as *C. cyanus*, it would clearly be less efficient in enhancing *M. mediator* in the field because it is not a suitable food source (Géneau et al. 2012). *Fagopyrum esculentum* in contrast is a very good food source for *M. mediator*, and numerous laboratory

studies (Araj et al. 2011; Géneau et al. 2012; Lavandero et al. 2006; Nafziger and Fadamiro 2011; Winkler et al. 2006; Witting-Bissinger et al. 2008) and field studies (Hogg et al. 2011; Lavandero et al. 2005; Lee and Heimpel 2005, 2008; Platt et al. 1999; Scarratt et al. 2008) have demonstrated its value for enhancing natural enemies. However it is less attractive than both *C. cyanus* and *I. amara*, so it seems less efficient at attracting *M. mediator* in an environment where it is not the only flower available—which would be the case in a wildflower strip composed of multiple flowers or in/around cabbage fields where other weeds are growing. Additionally, *C. cyanus* is the only plant among those we tested that produces easily accessible extrafloral nectar (Winkler et al. 2009), which is generally a much longer-lived potential resource for beneficial insects than floral nectar (Koptur 2005). There are therefore good reasons to think that *C. cyanus* has the best potential among the different flowers tested in this study to promote *M. mediator* in the field. This highlights the importance of taking flower attractiveness into account in the choice of suitable wildflowers for conservation biological control.

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References

- Ammon HPT, Kelber O, Okpanyi SN (2006) Spasmolytic and tonic effect of Iberogast® (STW 5) in intestinal smooth muscle. *Phytomedicine* 13(Supplement 1):67–74
- Araj S-E, Wratten S, Lister A, Buckley H, Ghabeish I (2011) Searching behavior of an aphid parasitoid and its hyperparasitoid with and without floral nectar. *Biol Control* 57(2):79–84
- Baggen LR, Gurr GM (1998) The influence of food on *Copidosoma koehleri* (Hymenoptera: Encyrtidae), and the use of flowering plants as a habitat management tool to enhance biological control of potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Biol Control* 11(1):9–17
- Begum M, Gurr GM, Wratten SD, Nicol HI (2004) Flower color affects tri-trophic-level biocontrol interactions. *Biol Control* 30(3):584–590
- Berndt LA, Wratten SD, Hassan PG (2002) Effects of buckwheat flowers on leafroller (Lepidoptera: Tortricidae) parasitoids in a New Zealand vineyard. *Agric For Entomol* 4(1):39–45
- Bianchi FJJA, Wäckers FL (2008) Effects of flower attractiveness and nectar availability in field margins on biological control by parasitoids. *Biol Control* 46(3):400–408
- Blumstein DT, Daniel JC (2007) Quantifying behavior the JWatcher way. Sinauer Associates, Inc., Sunderland, MA, USA, p 211
- Bone NJ, Thomson LJ, Ridland PM, Cole P, Hoffmann AA (2009) Cover crops in Victorian apple orchards: effects on production, natural enemies and pests across a season. *Crop Prot* 28(8):675–683
- Borg-Karlson A-K, Valterová I, Nilsson LA (1993) Volatile compounds from flowers of six species in the family Apiaceae: Bouquets for different pollinators? *Phytochemistry* 35(1):111–119
- Desouhant E, Driessen G, Amat I, Bernstein C (2005) Host and food searching in a parasitic wasp *Venturia canescens*: a trade-off between current and future reproduction? *Anim Behav* 70(1):145–152
- Ellis JA, Walter AD, Tooker JF, Ginzel MD, Reagel PF, Lacey ES, Bennett AB, Grossman EM, Hanks LM (2005) Conservation biological control in urban landscapes: manipulating parasitoids of bagworm (Lepidoptera: Psychidae) with flowering forbs. *Biol Control* 34(1):99–107
- Fabre N, Urizzi P, Souchard JP, Frécharde A, Claparols C, Fourasté I, Moulis C (2000) An antioxidant sinapic acid ester isolated from *Iberis amara*. *Fitoterapia* 71(4):425–428
- Ferro DN, McNeil JN (1998) Habitat enhancement and conservation of natural enemies of insects. In: Pedro B (ed) Conservation biological control. Academic Press, San Diego, USA, pp 123–132
- Finch S, Thompson AR (1992) Pest of cruciferous crops. In: Mc Kinlay RG (ed) Vegetable crop pests. Mac Millan Press Ltd, London, UK, pp 87–138
- Flamini G, Cioni PL, Morelli I (2003) Use of solid-phase microextraction as a sampling technique in the determination of volatiles emitted by flowers, isolated flower parts and pollen. *J Chromatogr A* 998(1–2):229–233
- Géneau CE, Wäckers FL, Luka H, Daniel C, Balmer O (2012) Selective flowers to enhance biological control of cabbage pests by parasitoids. *Basic Appl Ecol* 13:85–93
- Griffiths GJK, Holland JM, Bailey A, Thomas MB (2008) Efficacy and economics of shelter habitats for conservation biological control. *Biol Control* 45(2):200–209
- Hogg BN, Bugg RL, Daane KM (2011) Attractiveness of common insectary and harvestable floral resources to beneficial insects. *Biol Control* 56(1):76–84
- Jonsson M, Wratten SD, Landis DA, Gurr GM (2008) Recent advances in conservation biological control of arthropods by arthropods. *Biol Control* 45(2):172–175
- Koptur S (2005) Nectar as fuel for plant protectors. In: Wäckers FL, van Rijn PCJ, Bruin J (eds) Plant-provided food for carnivorous insects: a protective mutualism and its applications. Cambridge University Press, Cambridge, UK, pp 75–108
- Kugimiya S, Uefune M, Shimoda T, Takabayashi J (2010) Orientation of the parasitic wasp, *Cotesia vestalis*

- (Haliday) (Hymenoptera: Braconidae), to visual and olfactory cues of field mustard flowers, *Brassica rapa* L. (Brassicaceae), to exploit food sources. *Appl Entomol Zool* 45:369–375
- Landis D, Menalled FD (1998) Ecological considerations in the conservation of effective parasitoid communities in agricultural systems. In: Pedro B (ed) Conservation biological control. Academic Press, San Diego, USA, pp 101–121
- Landis DA, Wratten SD, Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annu Rev Entomol* 45:175–201
- Lauro N, Kuhlmann U, Mason PG, Holliday NJ (2005) Interaction of a solitary larval endoparasitoid, *Microplitis mediator*, with its host, *Mamestra brassicae*: host acceptance and host suitability. *J Appl Entomol* 129(9–10): 567–573
- Lavandero B, Wratten SD, Shishehbor P, Worner S (2005) Enhancing the effectiveness of the parasitoid *Diadegma semiclausum* (Helen): movement after use of nectar in the field. *Biol Control* 34(2):152–158
- Lavandero B, Wratten SD, Didham RK, Gurr GM (2006) Increasing floral diversity for selective enhancement of biological control agents: a double-edged sword? *Basic Appl Ecol* 7(3):236–243
- Lee JC, Heimpel GE (2005) Impact of flowering buckwheat on Lepidopteran cabbage pests and their parasitoids at two spatial scales. *Biol Control* 34(3):290–301
- Lee JC, Heimpel GE (2008) Floral resources impact longevity and oviposition rate of a parasitoid in the field. *J Anim Ecol* 77(3):565–572
- Leius K (1960) Attractiveness of different foods and flowers to the adults of some Hymenopterous parasites. *Can Entomol* 92:369–376
- Letourneau DK, Altieri MA (1999) Environmental management to enhance biological control in agroecosystems. In: Bellows TS, Fisher TW (eds) Handbook of biological control: principles and applications of biological control. Academic Press, New York, USA, pp 319–354
- Li J, Yan F, Coudron TA, Pan W, Zhang X, Liu X, Zhang Q (2006) Field release of the parasitoid *Microplitis mediator* (Hymenoptera: Braconidae) for control of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in cotton fields in northwestern China's Xinjiang province. *Environ Entomol* 35(3):694–699
- Luo S, Li J, Liu X, Lu Z, Pan W, Zhang Q, Zhao Z (2010) Effects of six sugars on the longevity, fecundity and nutrient reserves of *Microplitis mediator*. *Biol Control* 52(1):51–57
- Mir Khan S (1999) Effectiveness of *Microplitis mediator* (HYM. : Braconidae) against its hosts *Agrotis segetum* and *A. ipsilon* (Lepidoptera: noctuidae). *Pak J Biol Sci* 2(2): 344–346
- Nafziger JTD, Fadamiro HY (2011) Suitability of some farm-scaping plants as nectar sources for the parasitoid wasp, *Microplitis croceipes* (Hymenoptera: Braconidae): effects on longevity and body nutrients. *Biol Control* 56(3): 225–229
- Naranjo SE, Ellsworth PC (2009) The contribution of conservation biological control to integrated control of *Bemisia tabaci* in cotton. *Biol Control* 51(3):458–470
- Patt JM, Hamilton GC, Lashomb JH (1999) Responses of two parasitoid wasps to nectar odors as a function of experience. *Entomol Exp Appl* 90(1):1–8
- Pfiffner L, Luka H (2000) Overwintering of arthropods in soils of arable fields and adjacent semi-natural habitats. *Agric Ecosyst Environ* 78(3):215–222
- Pfiffner L, Luka H, Schlatter C, Juen A, Traugott M (2009) Impact of wildflower strips on biological control of cabbage lepidopterans. *Agric Ecosyst Environ* 129(1–3):310–314
- Platt JO, Caldwell JS, Kok LT (1999) Effect of buckwheat as a flowering border on populations of cucumber beetles and their natural enemies in cucumber and squash. *Crop Prot* 18(5):305–313
- Ponti L, Altieri MA, Gutierrez AP (2007) Effects of crop diversification levels and fertilization regimes on abundance of *Brevicoryne brassicae* (L.) and its parasitization by *Diaeretiella rapae* (M'Intosh) in broccoli. *Agric For Entomol* 9(3):209–214
- R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Raguso RA, Schlumpberger BO, Kaczorowski RL, Holtsford TP (2006) Phylogenetic fragrance patterns in *Nicotiana* sections *Alatae* and *Suaveolentes*. *Phytochemistry* 67(17): 1931–1942
- Scarratt SL, Wratten SD, Shishehbor P (2008) Measuring parasitoid movement from floral resources in a vineyard. *Biol Control* 46(2):107–113
- Schäffler I, Balao F, Dötterl S (2012) Floral and vegetative cues in oil-secreting and non-oil-secreting *Lysimachia* species. *Ann Bot* 110(1):125–138
- Siekmann G, Keller MA, Tenhumberg B (2004) The sweet tooth of adult parasitoid *Cotesia rubecula*: ignoring hosts for nectar? *J Insect Behav* 17(4):459–476
- Takasu K, Lewis W (1996) The role of learning in adult food location by the larval parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). *J Insect Behav* 9(2):265–281
- Turlings TCJ, Davison AC, Tamò C (2004) A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol Entomol* 29(1):45–55
- Verdonk JC, Haring MA, van Tunen AJ, Schuurink RC (2005) ODORANT1 regulates fragrance biosynthesis in petunia flowers. *Plant Cell* 17(5):1612–1624
- Wäckers FL (2004) Assessing the suitability of flowering herbs as parasitoid food sources: flower attractiveness and nectar accessibility. *Biol Control* 29(3):307–314
- Winkler K, Wäckers FL, Bukovinszkyne-Kiss G, van Lenteren JC (2006) Sugar resources are vital for *Diadegma semiclausum* fecundity under field conditions. *Basic Appl Ecol* 7(2):133–140
- Winkler K, Wäckers FL, Kaufman LV, Larraz V, van Lenteren JC (2009) Nectar exploitation by herbivores and their parasitoids is a function of flower species and relative humidity. *Biol Control* 50(3):299–306
- Witting-Bissinger BE, Orr DB, Linker HM (2008) Effects of floral resources on fitness of the parasitoids *Trichogramma exiguum* (Hymenoptera: Trichogrammatidae) and *Cotesia congregata* (Hymenoptera: Braconidae). *Biol Control* 47(2):180–186

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Oliver Balmer is leading the Functional biodiversity research at FiBL, Frick, Switzerland (jointly with Dr Henryk Luka). This research integrates laboratory, semi-field and field experiments to improve the biological control of cabbage pests via the addition of suitable wildflower strips and/or companion plants.