Attraction of *Aphidius ervi* (Hymenoptera: Braconidae) and *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) to sweet alyssum and assessment of plant resources effects on their fitness

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ABSTRACT

The green peach aphid *Myzus persicae* (Sulzer) is one of the most economically important aphid species affecting crops worldwide. Since many natural enemies of this aphid have been recorded, biological control of this pest might be a viable alternative to manage it. Selected plant species in field margins might help to provide the natural enemies with food sources to enhance their fitness. This study aimed to investigate if sweet alyssum, *Lobularia maritima* (L.), is a potential food source for the parasitoid *Aphidius ervi* Haliday and the predator *Aphidoletes aphidimyza* (Rondani), and whether this flower could contribute to enhance the biological control of *M. persicae*. Volatiles produced by alyssum, with and without flowers, attracted both natural enemies. This attractiveness to alyssum flowers was disrupted when compared with peach shoots recently infested with a relatively low number of aphids. When aphids were absent, parasitoids exposed to alyssum survived longer than those that fed on a sugar solution or on water. In the case of the predator, alyssum flowers did not benefit longevity since the nectaries were inaccessible to females. However, our results provide evidence that *A. aphidimyza* would be able to feed on nectar if accessible. The floral resource did not improve the reproductive capacity of the two natural enemies, but the 10% sugar solution increased the egg load of the predator. Provision of other sugar resources, such as flowers with exposed nectaries and extra floral nectar may also be a viable option to improve the biological control of *M. persicae*.

KEYWORDS egg load, floral nectar, longevity, olfactory response, sweet alyssum

Introduction

The green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is one of the most economically important aphid affecting crops worldwide. It is extremely cosmopolitan and highly polyphagous and hosts are in more of 40 different plant families including many economically important crops (Blackman and Eastop 2007). The green peach aphid, is a severe pests of peach and nectarine, vegetable and greenhouse crops (Rabasse and van Steenis 1999, Blümel 2004, Barbagallo et al. 2007). In a recent survey conducted in the Ebro Valley (Spain), a very important area of peach and nectarine production, pest advisors ranked this aphid as one of the most important pest problems (authors' unpublished data). The survey also revealed that pest management is currently mainly achieved using insecticides.

Biological control might be a viable alternative to manage *M. persicae*. Several predators and parasitoids of this species have been recorded, and this entomofauna might play an important role in the reduction of the aphid population (Völkl et al. 2007). The parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) and the predator *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) are among the most important natural enemies of this pest (Rabasse and van Steenis 1999, Blümel 2004). These natural enemies have been recorded in spring in the production areas were orchards coexist with arable crops (Pons and Stary 2003, Miñarro et al. 2005, Pons et al. 2011) and both have been repeatedly found on *M. persicae* colonies in *Prunus* orchards early in spring (authors' unpublished data). However, *M. persicae* attacks *Prunus* sp. in spring when the population of natural enemies is still low and, therefore, effective biological control of this aphid is difficult to achieve. The inclusion of floral resources close to the orchards might help to enhance the biological control by providing natural enemies with nectar

65 and pollen as food sources, thereby contributing to increase their survival and
66 reproduction (Landis et al. 2000, Gurr et al. 2005).

67 Sweet alyssum, *Lobularia maritima* (L.) (Brassicaceae), is a Mediterranean perennial
68 plant that blooms uninterrupted for extended periods (approximately 10 months), with
69 a maximum in spring (Picó and Retana 2001). It is very attractive to natural enemies and
70 thus has potential as an insectary plant (Chaney 1998, Alomar et al. 2008, Hogg et al.
71 2011). Ribeiro and Gontijo (2017) demonstrated that sweet alyssum increase the
72 abundance of generalist predators and therefore reduce some pests, especially aphids.
73 Sweet alyssum intercropping is widely used in the Salinas Valley in the central coastal
74 area of California to control aphids in organic lettuce and broccoli crops (Brennan 2013,
75 2016). Under laboratory conditions, it can improve the longevity of *A. ervi* (Araj et al.
76 2006, Araj and Wratten 2013) and the survival, egg load, and fecundity of other braconid
77 parasitoids such as *Dolichogenidea tasmanica* (Cameron) and *Diaeretiella rapae*
78 (Mcintosh) (Hymenoptera: Braconidae) (Berndt and Wratten 2005, Araj and Wratten
79 2015). However, there is little information about the effect of alyssum flowers on the
80 reproduction of *A. ervi* and the biology of *A. aphidimyza.*

81 The aim of this study was to investigate if *L. maritima* is a potential food source for *A.*
82 *ervi* and *A. aphidimyza*, and can therefore contribute to enhance the biological control
83 of *M. persicae*. To do that, we investigate if both natural enemies are attracted to
84 blooming and non-blooming alyssum. We also tested the effects of alyssum on the
85 longevity and the reproductive potential of *A. ervi* and *A. aphidimyza* in order to
86 evaluate the contribution of this plant to the fitness of these species.

87

Materials and methods

Insects and plant material

Mummies of *A. ervi* and pupae of *A. aphidimyza* were obtained from Agrobio©. Adult emergence took place inside a climatic chamber at 22°C and 70 ± 10% RH, with a 16:8 (L: D) photoperiod. When mated females were required, males and females (< 24 h old) were kept together for 24 h. The green peach aphid, *M. persicae*, was reared in the climatic chamber at the same conditions mentioned above on tobacco plants (*Nicotiana tabacum* L.). Plants of tobacco, peach, and alyssum were grown in plastic pots with compost soil in the greenhouse inside a closed compartment to prevent any pest infestation. Prior to each experiment, plants were observed and none of them had pest presence or symptoms of pest damage.

Longevity, egg load, fertility, and fecundity experiments were conducted at 22°C and 70 ± 10% RH, with a 16:8 (L: D) photoperiod. Olfactometer assays were carried out at 22°C and 60 ± 10% RH under light conditions. A single lamp (Sylvania Circline FC22W/865) placed at 60 cm above the Y-tube was used. These light conditions were set up because *A. ervi* emerge during the photophase (He et al. 2004) and *A. aphidimyza* emerge before sunset (Harris 1973).

Olfactory bioassays

Experiments with *A. ervi* and *A. aphidimyza* were conducted in a Y-tube olfactometer. Each arm was 17 cm long and had a diameter of 3.5 cm; the inside angle between the two closest arms was 75°. Each of these two arms received air from one of the two odor sources that were inside two glass jars (4000 mL) connected to them. The air coming

from a compressor (ABAC-FC2-24CM) passed through a double carbon filter (ABAC-ACF60 1000 Lmh) and an air humidifier (water bubbler) and subsequently entered the glass jars. Air flow was adjusted to 0.20 ± 0.03 m/s at the base of the third arm of the olfactometer and was measured with a hot-wire anemometer (Testo, Barcelona, Spain). Insects were gently placed at the base of the main arm and allowed to move in. They were considered to make a choice when they walked more than 5 cm on one of the upper arms in less than 10 min. To avoid any possible asymmetries in the experimental set-up due to environmental factors or location effects, after five individuals, the olfactometer was cleaned with alcohol (96%) and the arms were switched between the two odor source jars. Jar positions were also rotated after every 10 female adults. Forty female parasitoids and predators (1 to 4-days-old) were individualized and starved for 24 h prior to each observation. Each individual was used only once. In the case of *A. ervi,* the position of the olfactometer was vertical, whereas for *A. aphidimyza*, it was horizontal. The position for each species was proposed after preliminary tests. The following choices were offered to *A. ervi* and *A. aphidimyza*: (1) alyssum flowers *vs.* clean air, (2) alyssum plant without flowers *vs.* clean air, (3) alyssum flowers *vs.* alyssum plant without flowers, (4) aphid-free peach shoots *vs.* alyssum flowers, 5) aphid-infested peach shoots *vs.* alyssum flowers. In the treatments with blooming alyssum, three shoots, which together had about 40 fully open alyssum flowers, were used; in the case of non-flowering alyssum, three shoots with only green leaves were used. To infest peach shoots with aphids, 24 h prior to the experiment, approximately 50 second to third-instar *M. persicae* were placed gently onto the leaves with a brush. All plant shoots were cut just before the start of the experiment. The cut end was immediately submerged in water in a jar with a bored lid. The stems were introduced in the hole

which was closed with a piece of paper to prevent wound-related volatiles during the olfactory assay. Each day, new plant material and aphids were used.

Effects of alyssum and sugar solution on the biology of *A. ervi*

Female longevity

Females of *A. ervi* less than 24 h old were placed individually in a 250 mL plastic cup covered with gauze to provide ventilation. We tested three different food sources: 1) a 70% sugar rich diet solution of glucose, fructose, and sucrose (G + F + S) in a 1:1:1 ratio, 2) three shoots of alyssum with approximately a total of 40 fully open flowers, and 3) water as control. The above-mentioned sugars were chosen because they are the main components of the nectar (Baker and Baker 1983, Wackers 2001) and a 70% sugar solution supports a longer lifetime of *A. ervi* females (Azzouz et al. 2004). The three diets were offered to *A. ervi* females in the presence and in the absence of aphids, resulting in six different treatments. Sugar solution and water were provided in a 13-mL tube plugged with a piece of cotton dental roll and attached to the wall of the cup with Blue-tack® (Rubi, Spain). Alyssum flowers were kept in an Eppendorf vial with water and also attached to the glass wall with Blue-tack®. In the treatments with aphids, 20 second to third-instar *M. persicae* were placed on the top of a tobacco disc that was laid above an agar layer (0.5%) on a 2.5 cm Petri dish which was introduced on the base of the cup. Food and aphids were renewed twice per week. Female mortality was recorded daily. Fifteen replications were performed per treatment.

Egg load and fertility

To evaluate egg load, females (< 48h old) were caged for three days in arenas without aphids similar to those described in the previous section (*A. ervi* longevity) and subsequently frozen at -20°C until dissection. To do that, the females were placed on a microscope slide under a stereomicroscope. With a scalpel the thorax was separated from the abdomen, that was subsequently open to remove the ovaries and the number of chorionated oocytes recorded. The effect of the same food treatments on fertility were evaluated in arenas with aphids as prepared for *A. ervi* longevity. Tobacco discs with aphids and food were renewed every three to four days. Aphid mortality was assessed in the discs when removed from the cups. Aphids that did not move their legs when touched with a fine brush were considered dead (see Moores et al. 1996). Subsequently, the tobacco discs were kept in the climatic chamber at 22°C until the aphids were mummified. Fifteen leaf discs with aphids, but without parasitoids, were prepared to assess natural and handling mortality. The results were used to correct mortality produced by the parasitoids.

Effects of alyssum and sugar solutions on the biology of *A. aphidimyza*

Female longevity

Starved females less than 24 hours old were isolated in arenas without aphids similar to those used in the *A. ervi* longevity trials. Instead of 250 mL plastic cups, glass cups were used. A 10% G + F + S solution was provided as sugar-rich diet according to the findings of Watanabe et al. (2014). Mortality was checked daily.

177 **Egg load and fecundity**

178 The same methodology as described for the experiment to assess *A. ervi* egg load, but
179 using glass cups and a 10% instead of a 70% G + F +S solution, was applied to evaluate
180 the effects of a sugar-rich diet on *A. aphidimyza* egg load and fecundity. Egg load was
181 determined by dissecting the abdomen of the females as explained above for *A. ervi*. For
182 fecundity, the number of eggs laid on the aphid colony on the leaf were counted daily.
183 Twenty females were tested per treatment.

184 **Survival up to five days**

185 A specific experiment was carried out to check if starved *A. aphidimyza* females less than
186 24 h old were able to feed on alyssum nectar. We used the same set up without aphids
187 described when assessing *A. ervi* longevity. However, a fourth type of food, alyssum
188 flowers with plucked petals and thereby exposed nectaries, was included. Survival was
189 measured up to five days, with five replications per treatment.

190 **Morphometry of *A. aphidimyza* and alyssum flowers**

191 After the longevity trial, several visual observations were made to record how females
192 approached the nectaries and how the insects placed themselves on the flower for
193 feeding. To do this, we used one to four-day-old female predators that were starved for
194 24 hours prior to each observation. Individuals were released in Petri dishes containing
195 alyssum flowers and we recorded the time spent by females from landing on the flowers
196 until they walked away with a timer. After these observations, we measured the gap
197 between the petals and the stamen of alyssum flowers as well as the distance between

198 the femur and tibia intersection points of both middle legs. All measurements were
199 made with a dissection microscope at 2.5 x magnification, using the program ImageJ.

200 ## Data analysis

201 Differences in the proportion of *A. ervi* and *A. aphidimyza* females choosing a particular
202 odor source (olfactometer experiments) were tested using a two-sided binominal test.
203 Insects that did not respond within 10 minutes were not included in the analysis. Data
204 of *A. ervi* longevity in the arenas with aphids, *A. aphidimyza* longevity, the egg load of
205 both natural enemies, *A. aphidimyza* fecundity, and the total number of mummies and
206 dead aphids in the trials with the parasitoids were analyzed by one-way ANOVA; means
207 were separated using Tukey´s HSD test. Since data of *A. ervi* longevity in the arenas
208 without aphids could not be normalized, a Kruskal-Wallis test was used in the analysis
209 and Mann-Whitney-Wilcoxon tests were used to observe pair-wise differences between
210 treatments with Bonferroni-weighted test correction ($P<0.05$). Survivorship affected by
211 diet was evaluated using the Kaplan-Meier survival platform. Pairwise comparisons
212 among groups were evaluated using log-rank tests with α set at 0.005 to account for
213 multiple comparisons. All data were analyzed using SAS 9.3 for Windows; survival curves
214 were generated with the software SigmaPlot version 13.

215 # Results

216 ## Olfactory bioassays

217 Significantly more *A. ervi* females preferred alyssum, either with or without flowers, to
218 clean air (Figure 1), whereas they showed no significant preference for any treatment
219 when offered a choice between alyssum shoots with and without flowers. The volatiles

from alyssum flowers were significantly more attractive than those from the peach shoots without aphids. When alyssum flowers were compared to the peach shoots with aphids, the parasitoids did not show a significant preference for any of them. The mean time that an *A. ervi* females spent to respond to the odor source ranged from 53 to 103 s.

Aphidoletes aphidimyza females significantly preferred alyssum shoots, either with or without flowers, to clean air (Figure 2). When *A. aphidimyza* females were offered a choice between alyssum shoots with and without flowers, they showed a significant preference for the blooming alyssum. Likewise, predators significantly preferred cues from alyssum flowers to those of the clean peach shoots, but they did not display a significant preference between alyssum flowers and peach shoots infested with aphids. The mean time spent by an *A. aphidimyza* female to respond to the cues ranged from 111 to 163 s.

Effects of alyssum and sugar solution on the biology of *A. ervi*

Female longevity

The mean longevity of *A. ervi* females in the treatments with different diets and with and without aphids is presented in Figure 3. There was an interaction between longevities recorded in the arenas with and without aphids and, therefore, data were analyzed separately. When aphids were present in the arenas, longevity was not significantly different, regardless of the food treatment ($F_{2, 42} = 0.29$, $P = 0.74$). In contrast, when aphids were absent, longevity significantly varied among food sources ($\chi^2 = 21.22$, $P < 0.0001$). Females which fed on alyssum significantly lived longer than

242 those which fed on the sugar solution or water (Z = 2.50, P = 0.0122; Z = 4.38, P < 0.0001,
243 respectively; Mann-Whitney test, Bonferroni corrected significance p-value < 0.0167 =
244 0.05/3). The longevity of females which fed on the sugar solution was also significantly
245 higher than that of females which fed on water (Z = 2.44, P = 0.143 Mann-Whitney U-
246 test, Bonferroni corrected significance p-value < 0.0167 = 0.05/3).

247 There was no significant difference in the survival curves of individuals fed with different
248 food sources in the presence of aphids (Log-rank χ^2= 5.59, *df= 2,* P = 0.060) (Fig. 4A).
249 Survival curves differed significantly between food sources in the absence of aphids
250 (Log-rank χ^2= 25.43, *df= 2,* P < 0.0001) (Fig. 4B).

251 **Egg load and fertility**

252 After 72 h of feeding on different food sources, all *A. ervi* females had 0 or 1 mature
253 oocyte when dissected, and no significant differences were observed in the egg load ($F_{2,}$
254 $_{42}$ = 0.82, P= 0.44). Table 1 shows the total number of dead aphids corrected by natural
255 and handling mortality (3.75 ± 0.37 individuals). No significant differences were
256 observed among different foods, neither in the number of mummies nor in the number
257 of dead aphids ($F_{2, 39}$ = 0.38, P= 0.68 and $F_{2, 39}$ = 0.48, P = 0.62, respectively).

258 ## Effects of alyssum and sugar solutions on the biology of *A. aphidimyza*

259 **Female longevity**

260 The survival curve showed significant differences among food sources (Log-rank χ^2 =
261 34.54, *df*= 2, P < 0.0001) (Fig. 5). Total longevity of *A. aphidimyza* females significantly
262 varied among food sources ($F_{2, 42}$ =37.66, P < 0.0001). Significantly longer longevity was
263 recorded for females which fed on the sugar solution (8.1 ± 0.62 days) than for unfed

ones (4.2 ± 0.20 days) and those provided with alyssum flowers (3.3 ± 0.30 days). No significant differences were observed in the longevity of individuals fed with the two latter food sources.

Egg load and fecundity

Diet significantly affected *A. aphidimyza* egg load ($F_{2, 57} = 5.22$, $P < 0.05$). The number of mature oocytes was significantly higher when females fed on a 10% G + F + S solution than on water or on intact alyssum flowers (Table 2). There was no significant difference between females fed with alyssum and unfed ones. Daily oviposition rates were not significantly different between the three treatments ($F_{2, 42} = 0.67$, $P = 0.51$).

Survival up to five days.

Significant differences in survival after five days feeding on different foods were recorded for *A. aphidimyza* ($F_{3, 16} = 45.36$, $P < 0.0001$). Females fed on the sugar solution and on exposed alyssum nectaries survived significantly longer that those fed on intact alyssum flowers or water (Fig. 6).

Morphometry of *A. aphidimyza* and alyssum flowers.

Our observations revealed that predator females had difficulties to reach alyssum nectar glands, and none of the 10 observed females contacted the nectaries. They were observed on the top of the flowers lowering their head to try to reach the nectar glands at the very bottom inside the corolla tube (Fig. 7). The females spent a mean time of 42.3 s (± 6.45) on the petals and then left the flowers. Measurements indicated that the distance between the two joints of the femur with the tibia in middle legs of

285 *A. aphidimyza* females is wider (1.49 ± 0.12 mm) than the gap between petals and
286 stamen of the flowers (0.27 ± 0.04 mm) (Fig. 8).

287 Discussion

288 In our olfactometer experiments, *A. ervi* and *A. aphidimyza* were attracted to flowering
289 and non-flowering alyssum. According to (Harris 1973) *A. aphidimyza* is nocturnal.
290 However, females responded to the cues emitted by alyssum under light conditions.
291 Possibly, they may also locate the plants during the scotophase since many of them
292 produce volatiles at night (Kumari et al. 2017). Attraction to blooming alyssum in field
293 and laboratory studies is well documented for some natural enemies as predators and
294 some braconid parasitoids (Foti et al. 2017; Gontijo et al. 2013; Arnó et al. 2012; Rohrig
295 et al. 2008; Alomar et al. 2006). The similar attraction between flowering and non-
296 flowering alyssum has been also reported for the parasitoid *Trissolcus basalis*
297 (Wollaston) (Hymenoptera: Platygastridae) (Foti et al. 2017). Interestingly, this
298 attractiveness to alyssum flowers was disrupted when compared with peach shoots
299 recently infested with a relatively low number of aphids (50 individuals during 24 hours),
300 similarly to what has been reported for *A. ervi* by Guerrieri et al. (1999). This indicates
301 that volatiles produced by aphid-infested plants (Guerrieri et al. 1993, Reed et al. 1995,
302 Du et al. 1997, Hou et al. 1997, Powell et al. 1998, Desurmont et al. 2015), by the
303 honeydew (Budenberg and Powell 1992, Du et al. 1997, Choi et al. 2004,
304 Wickremasinghe 2007), and/or by the aphids themselves (Reed et al. 1995, Du et al.
305 1996) were attractive enough to balance the attraction produced by alyssum flowers.
306 Our results suggest that both natural enemies are able to rapidly locate aphid colonies,
307 which would benefit the effectiveness of these two natural enemies. Since the amounts
308 of volatiles produced by the plant/aphid complex will increase with time as the aphid

309 colonies increase in size, attraction of *A. ervi* and *A. aphidimyza* to the aphid-infested
310 plants will probably increase, as has been demonstrated for *Aphidius gifuensis*
311 (Ashmead) (Hymenoptera: Braconidae) (Yang et al. 2009).

312 Our results also indicate that the presence of additional sugar-rich-food was relevant in
313 terms of *A. ervi* survival only when aphids were not present in the arena. In that case,
314 alyssum nectar increased female longevity compared to that of unfed ones and was even
315 a better food source for *A. ervi* than a sugar solution containing glucose, fructose, and
316 sucrose, which are the main sugars present in nectar (Wackers 2001, Winkler et al.
317 2005). Higher longevity of parasitic wasps feeding on alyssum compared to sugar-fed
318 individuals has been shown before for *A. ervi* (Wade and Wratten 2007, Araj et al. 2006,
319 Araj and Wratten 2013). These higher survival suggested that besides of sugars, other
320 food substances (such as amino acids, lipids, proteins, vitamins, and minerals) present
321 in flowers even in small quantities play an important role in the longevity of *A. ervi*
322 females (Baker and Baker 1983, Wackers 2005). Pollen is unlikely to be a food resource
323 used by parasitoids (Jervis 1998, Irvin et al. 2006).

324 On the other hand, when aphids were present, the provision of additional resources did
325 not increase *A. ervi* longevity, suggesting that the combination of honeydew and hosts
326 is adequate to keep females alive. In fact, several studies have shown that parasitoids,
327 including *A. ervi*, are well adapted to the use of insect-produced honeydew which is the
328 predominant sugar source in many agricultural systems (Burger et al. 2004, Lenaerts et
329 al. 2016).

330 In our experiments done in the absence of aphids the maximum egg load recorded for
331 *A. ervi* females after feeding for 72 h was one mature oocyte, regardless of the food

treatment. This was probably due to the reabsorption of mature oocytes when hosts were not available since this species is pro-synovigenic and females emerge with approximately 20 to 60 mature eggs (He and Wang 2006). This reabsorption has been described to occur within 48-72 hours following emergence in other braconids such as *D. rapae* (Kant et al. 2013). When aphids were available, the number of mummies was the same, regardless of the food, indicating a similar fertility. This also implies that an additional food source is not required when the host and the honeydew are present. Similar results have been observed by Hayashi and Nakashima (2014), who found that for *A. ervi*, female progeny did not differ between unfed females and those fed with a sugar solution.

Our experiments show that in the absence of aphids, a sugar-rich diet benefited *A. aphidimyza* female longevity and egg load, similarly to what has been observed by Watanabe et al. (2014). On the contrary, the presence of alyssum flowers did not enhance the survival or the number of mature oocytes of *A. aphidimyza* females, probably because nectar was not accessible for them, as was confirmed when alyssum flowers with exposed nectaries were offered. Our results provide evidence that *A. aphidimyza* would be able to feed on nectar if it was accessible and, therefore, it may explain why females were attracted to alyssum flowers. To our knowledge, there are no records in the literature of *A. aphidimyza* females feeding on floral resources.

Our observations and the measurements performed on both the flower and *A. aphidimyza* confirmed that females could not access the very bottom part inside the corolla of alyssum flowers where the nectaries are found (Patt et al. 1997). According to our results, this was due to their long legs and the large span between the femur and

355 tibia joints of both middle legs, which was wider than the gap between the petals and
356 the stamen of the flower and thereby prevented access to the nectar. In addition,
357 females were not strong enough to separate the flower structures. Similar results has
358 been observed in some parasitoids (Rabb and Bradley 1968, Jervis et al. 1993, Patt et al.
359 1997, Rahat et al. 2005) and some predators (Nave et al. 2016, van Rijn and Wackers
360 2016).

361 On the other hand, *A. aphidimyza* daily fecundity was similar regardless of the additional
362 food supplied. This was probably due to the same amount of aphids present in all the
363 treatments, that is to say the same aphid density, which influenced the amount of
364 honeydew, a good food resource for this predator (El-Gayar 1976, Sell and Kuo-Sell
365 1987, Choi et al. 2004). Fecundity of *A. aphidimyza* strongly depends on the aphid
366 density in both laboratory experiments (Choi et al. 2004, Guo et al. 2014) and field
367 studies (Stewart and Walde 1997, Sentis et al. 2012).

368 In conclusion, both natural enemies of *M. persicae*, the parasitoid *A. ervi* and the
369 predator *A. aphidimyza,* were attracted to alyssum plants. Therefore, the establishment
370 of crop margins including this plant species, that is fully blooming in spring (Picó and
371 Retana 2001), may help to attract these naturally occurring beneficials in the area (Pons
372 and Stary 2003, Miñarro et al. 2005, Pons et al. 2011) into orchards and, increase their
373 local population regardless of the presence of aphids. The presence of alyssum flowers
374 close to the fields would increase *A. ervi* longevity and probably their ability for host
375 searching as soon as aphid populations start to build up. This beneficial effect for the
376 parasitoid would not be relevant with high *M. persicae* populations because at this point
377 it may obtain nutrients from honeydew. In the case of *A. aphidimyza* and due to the

inaccessibility of alyssum nectar for the adults, these flowers will not represent a supplemental food for the females. Therefore, to consider nectar accessibility while selecting insectary plants is important because attracting insects without providing accessible nectar, and therefore additional energy, may be detrimental and most likely results in inadequate energy use (Winkler et al. 2009). Because of that the combination of alyssum flowers and flowers with exposed nectaries or plants with extra floral nectar may also be viable options to improve the biological control of *M. persicae*. Other food sources such as honeydew of non-pest aphids or sugar provision via dispensers may also be useful to enhance natural enemy fitness. Further field experiments will be necessary to fully understand the potential role of different sugar-rich diets in the biological control of aphids in peach orchards.

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595 **Fig. 1.** Number of *A. ervi* female attracted to different treatments in a Y-tube
596 olfactometer (total number of females tested = 40). The Z and P values relate to a two-
597 sided binomial test of observed and predicted distribution based on a random
598 response.*Indicate significant differences between treatments. Individuals that did not
599 respond were not included in the analysis. The mean (± SE) response time from top to
600 bottom were 61.19 ± 8.34, 52.94 ± 5.98, 83.84 ± 8.57, 103.28 ± 18.54 and 55.40 ± 9.64
601 s.

602 **Fig. 2.** Number of *A. aphidimyza* female attracted to different treatments in a Y-tube
603 olfactometer (total number of females tested = 40). The Z and P values relate to a two-
604 sided binomial test of observed and predicted distribution based on a random
605 response.*Indicate significant differences between treatments. Individuals that did not
606 respond were not included in the analysis. The mean (± SE) response time from top to
607 bottom were 133.87 ± 19.51, 115.23 ± 7.81, 110.89 ± 5.30, 110.79 ± 23.06, 163.22 ±
608 18.87 s.

609 **Fig. 3**. Mean longevity of *A. ervi* females with three different diets in two scenarios, with
610 and without aphids. Different upper-case letters indicate no differences among
611 treatments in the presence of aphids (ANOVA $P < 0.05$). Lower-case letters indicate
612 differences among treatments in the absence of aphids (Mann-Whitney U-tests with
613 Bonferroni correction; a value of $p < 0.0167$ was considered statistically significant.
614 There was an interaction between longevities in the case of alyssum. G+F+S stands for a
615 70% sugar rich water solution of glucose, fructose, and sucrose in a 1:1:1 ratio.

Fig. 4. Kaplan-Meier estimates of survivorship functions of *A. ervi* females given access to water (control), 70% sugar water solution of glucose, fructose, and sucrose in a 1:1:1 ratio (G + F + S), and alyssum flowers in the presence (A) and in the absence (B) of aphids.

Fig. 5. Kaplan-Meier estimates of survivorship functions of *A. aphidimyza* females given access to water (control), 10% sugar water solution of glucose, fructose, and sucrose in a 1:1:1 ratio (G + F + S), and alyssum flowers.

Fig. 6. Number of days *A. aphidimyza* females survive, up to five days, when provided with different foods. Different letters indicate significant differences between the food treatments (ANOVA, Tukey´s HSD for mean separation, $P < 0.05$).

Fig. 7. Lateral view of *A. aphidimyza* on alyssum flower, showing the nectar glands position (black dots) inside the calix.

Fig. 8. Ventral view of an *A. aphidimyza* female (A) and above view of an alyssum flower (B). Comparison of the measures between the joint of the femur and tibia in the middle legs of *A. aphidimyza* (14.9 ± 1.2 mm, mean ±SE) and the gap between the petals and the stamens of the alyssum flower (0.27 ± 0.04 mm, mean ± SE).

631 **Table 1.** Mean (± SE) number of *A. ervi* mummies and dead aphids (± SE) when female
632 wasps were fed with three different treatments. No significant differences were found.

Treatment	Mummies (mean ± SE)	Dead aphids (mean ± SE)
Water	8.88 ± 1.55	21.73 ± 3,11
Alyssum	11.00 ± 1.96	25.67 ± 3.58
G + F + S 70%	10.28 ± 1.74	22.08 ± 2.65

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634

635 **Table 2.** Mean number (± SE) of mature oocytes inside *A. aphidimyza* females and eggs
636 laid per day when fed with three different treatments.

Treatment	Oocytes (mean ± SE)	Eggs /day (mean ± SE)
Water	27.25 ± 4.94b	5.16 ± 1.64a
Alyssum	27.55 ± 3.98b	2.73 ± 0.96a
G + F + S 10%	43.90 ± 3.43a	3.68 ± 1.77a

637 Different letters in the same column indicate significant differences (ANOVA, Tukey´s
638 HSD for mean separation, $P < 0.05$).
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Fig.1.

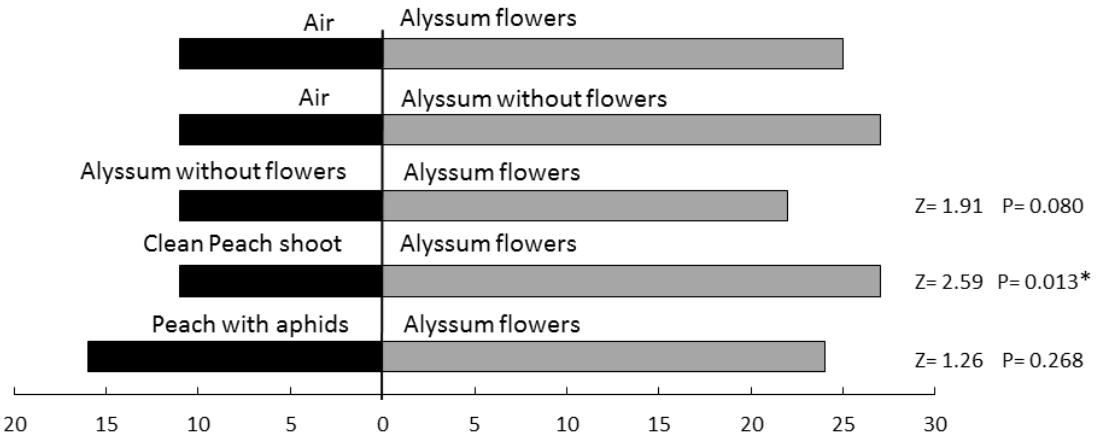
Air
Alyssum flowers
Air
Alyssum without flowers
Alyssum without flowers
Alyssum flowers
Z= 1.91 P= 0.080
Clean Peach shoot
Alyssum flowers
Z= 2.59 P= 0.013*
Peach with aphids
Alyssum flowers
Z= 1.26 P= 0.268
20 15 10 5 0 5 10 15 20 25 30

Fig.2.

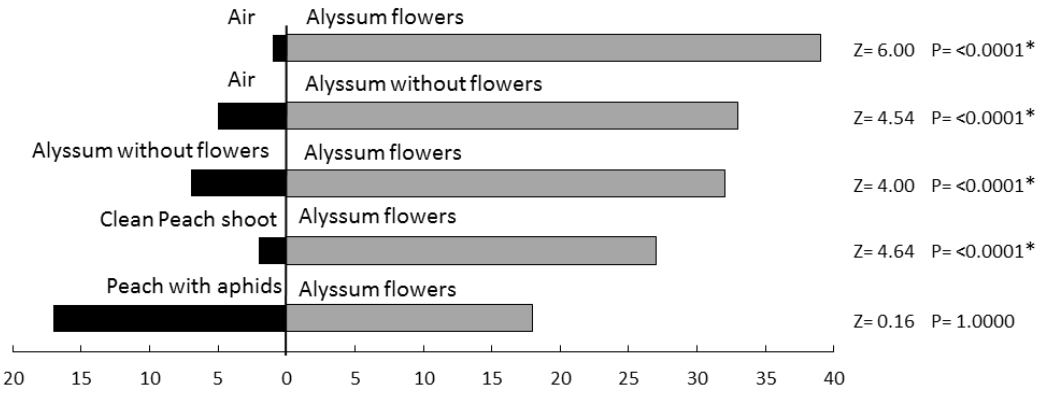
Air
Alyssum flowers
Z= 6.00 P= <0.0001*
Air
Alyssum without flowers
Z= 4.54 P= <0.0001*
Alyssum without flowers
Alyssum flowers
Z= 4.00 P= <0.0001*
Clean Peach shoot
Alyssum flowers
Z= 4.64 P= <0.0001*
Peach with aphids
Alyssum flowers
Z= 0.16 P= 1.0000
20 15 10 5 0 5 10 15 20 25 30 35 40

Fig. 3.

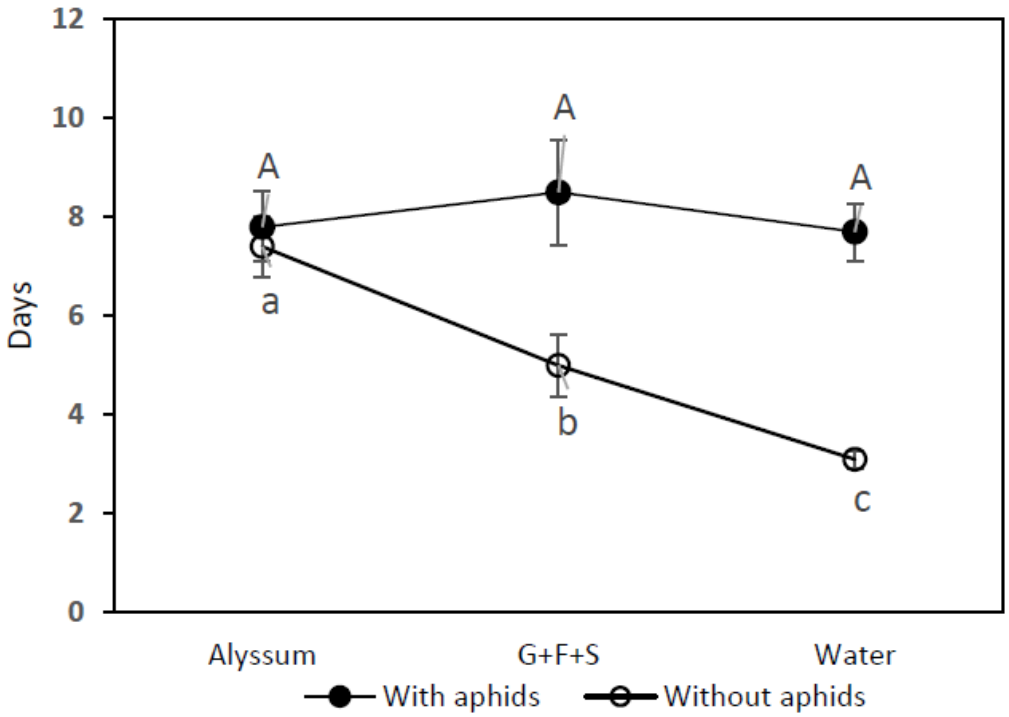
12
10
8
6
4
2
0
Days
A
A
A
a
b
c
Alyssum
G+F+S
Water
With aphids
Without aphids

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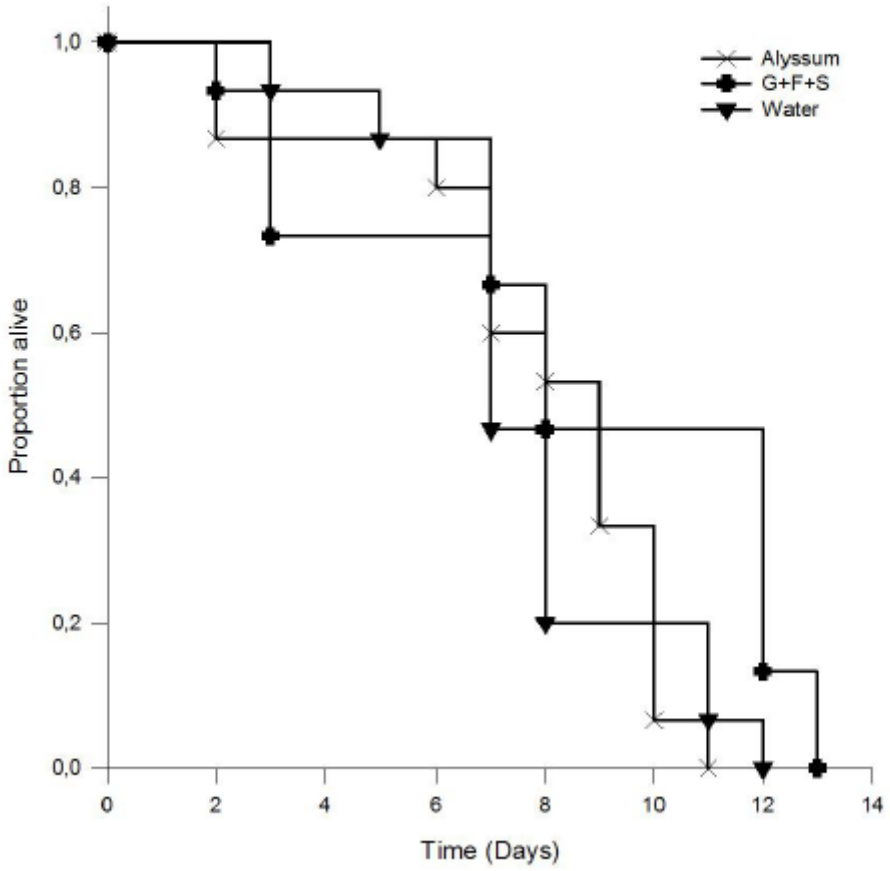
Fig. 4.

A)


Alyssum
G+F+S
Water
Proportion alive
1,0
0,8
0,6
0,4
0,2
0,0
0
2
4
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12
14
Time (Days)

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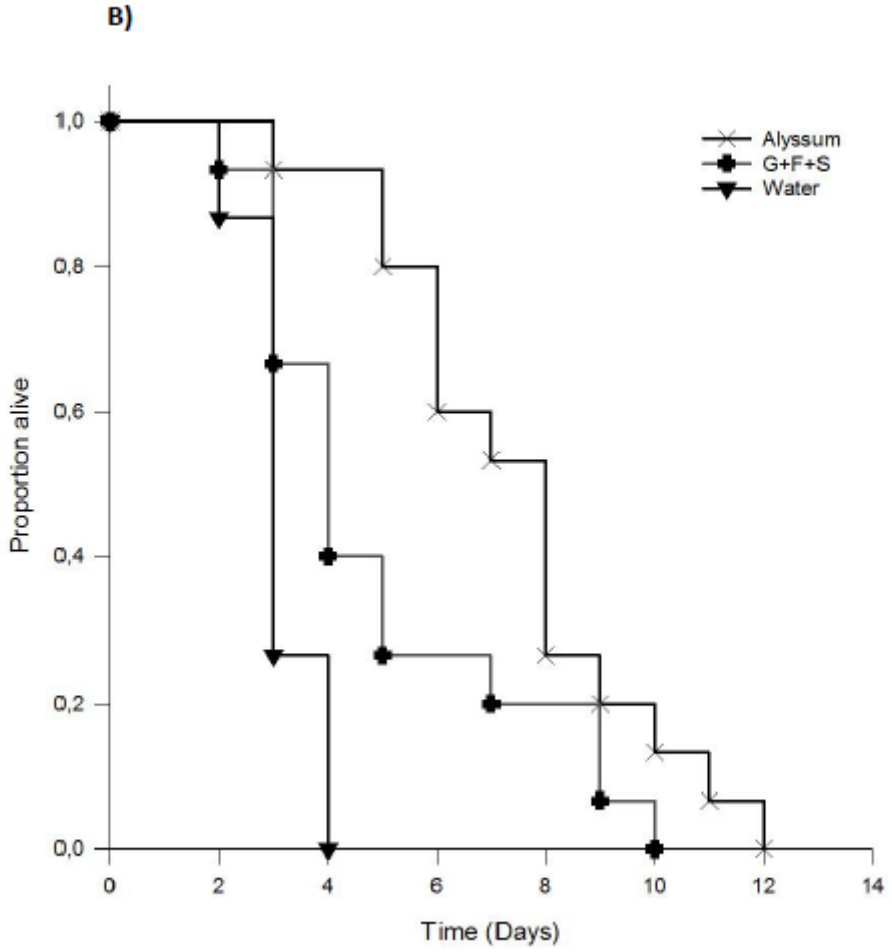

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Fig. 5.

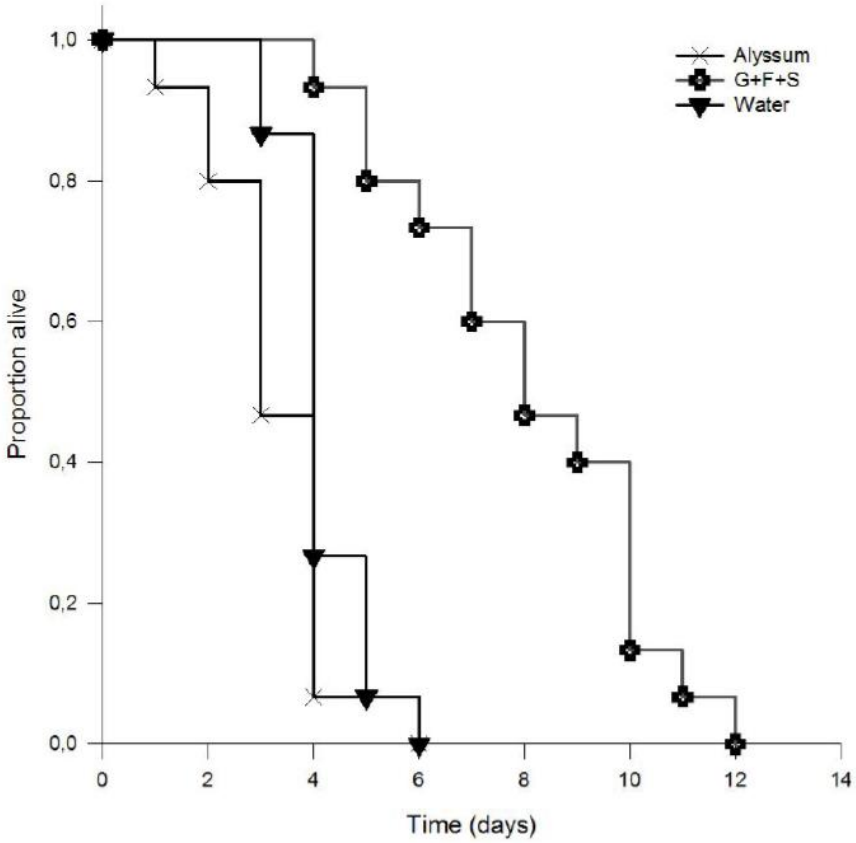

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Fig. 6.

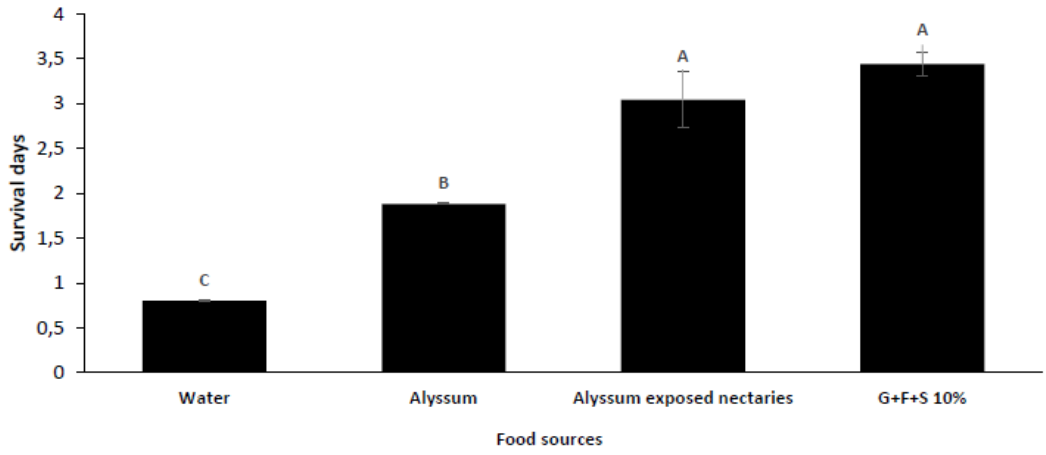

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Fig. 7.

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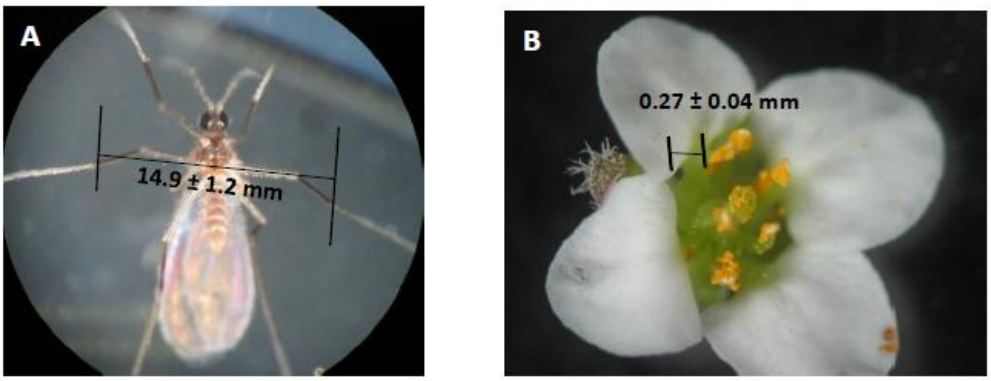
Fig. 8.



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