



## Flowers promote aphid suppression in apple orchards

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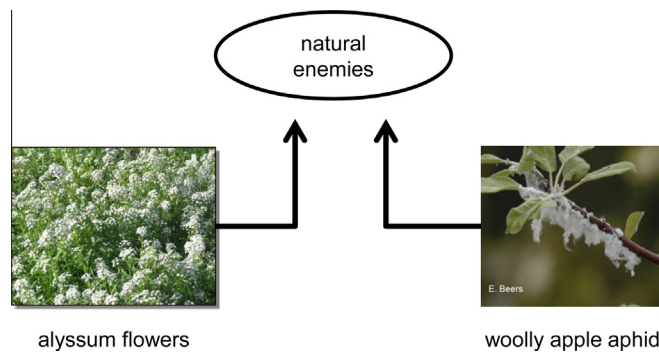
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### HIGHLIGHTS

- Sweet alyssum flowers had the highest attractiveness to syrphids.
- Faster suppression of woolly apple aphid occurred on trees closer to alyssum flowers.
- Higher densities of natural enemies were observed near sweet alyssum plantings.
- Natural enemies were found to move between sweet alyssum and adjacent apple trees.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Predators and parasitoids often benefit from feeding on nectar and/or pollen, such that the addition of flowering plants should bolster natural enemies and improve biological control. Nonetheless, this conceptually-simple approach often fails to reduce pest numbers. We examined whether flowering annual plants drew more natural enemies to apples (*Malus domestica*) in Washington State, USA, and in turn whether this improved suppression of woolly apple aphids (*Eriosoma lanigerum*) on nearby trees. Initial screening of candidate flowers indicated that syrphid flies (Diptera: Syrphidae), thought to be important aphid predators, were particularly attracted to sweet alyssum flowers (*Lobularia maritima*). Therefore, in two subsequent field experiments we compared aphid densities on trees placed adjacent to, or relatively far from, flowering sweet alyssum. The results were striking: after one week aphid densities were significantly lower on trees adjacent to flowers than on those on control plots, and these differences were maintained for several weeks. It was unlikely that aphid decline was primarily due to syrphid predation, because lower aphid densities were observed despite few syrphid larvae being present. Rather, a diverse group of generalist-predator spiders and bugs increased significantly near sweet alyssum plantings, and may have been responsible for much of the aphid suppression that we observed. Immunomarking revealed that natural enemies regularly moved from sweet alyssum to the surrounding orchard. In summary, the floral resources that sweet alyssum plants provided attracted natural enemies and indirectly suppressed densities of woolly apple aphids, suggesting an effective means for apple growers to enhance biological control.

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### 1. Introduction

Predators and parasitoids of herbivorous pests often benefit from the presence of flowering plants, when the natural enemies

can use pollen or nectar as supplemental food (Landis et al., 2000). For example, lady beetles inhabiting alfalfa fields have been observed to use pollen as alternative food source, which may help bolster predator numbers and their impacts on aphid and beetle pests (Davidson and Evans, 2010). Likewise, adult female parasitoids can dramatically increase their lifespan, and thus net fecundity, when provided with access to nectar from flowers (Winkler et al., 2006). The addition of these “floral resource” to

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agroecosystems could be one obvious means to enhance biological control of agricultural pests, by planting annual or perennial flowering plants at field edges (Hickman and Wratten, 1996). In several cases this approach has been notably successful. For example, Tylianakis et al., (2004) observed a significant increase in aphid parasitism in wheat plots closer to floral-resource patches.

Nonetheless, there are surprisingly few published case-studies where the addition of flowering plants has both enhanced natural enemy populations and improved pest suppression (Heimpel and Jervis, 2005). There are many reasons why adding flowering plants might fail to improve biological control. First, the flowers must add novel resources not already provided elsewhere in the environment by aphid honeydew or other sugar sources. For example, the addition of new floral resource may not reliably improve parasitoid performance when sugars from aphid honeydew are already available (Lee et al., 2006). Second, flowers must be carefully chosen to provide resources easily accessed by natural enemies but not by moths or other pests that also use nectar or pollen (Baggen and Gurr, 1998; Baggen et al., 1999; Géneau et al., 2012), or by intraguild predators or parasitoids of the natural enemy species that one is seeking to conserve (Araj et al., 2006; Prasad and Snyder, 2006; Jonsson et al., 2009). Third, natural enemies must readily move back and forth between flowers and the crop which one is seeking to protect, and not simply aggregate and arrest in the flowers (Landis et al., 2000). These obvious limitations have led some authors to question how often the provisioning of floral resources is likely to enhance, rather than disrupt (or simply fail to alter), overall biological control (Heimpel and Jervis, 2005).

We examined the potential for annual flowering plants to attract natural enemies that attack woolly apple aphids (*Eriosoma lanigerum*) in Washington State, USA, apple (*Malus domestica*) orchards. In our region the aphids typically reach high densities soon after bud-break, and then again later in the growing season starting in July; peak aphid densities are often seen between July and October (Beers et al., 2010). The relationship between aphid density and yield reduction is not known, such that growers usually reach spray decisions in the absence of rigorously-documented action thresholds (Beers et al., 2010). There have been increasingly severe woolly apple aphid outbreaks in our region in recent years (Beers et al., 2010). It is likely, that in the past aphids were indirectly killed by sprays of organophosphate and other broad spectrum insecticides targeting codling moth (*Cydia pomonella*). In addition, mating disruption and reduced-risk alternative management programs for the primary pest have been associated with higher aphid densities.

Woolly apple aphids are attacked by the specialist parasitoid *Aphelinus mali*, and also preyed upon by syrphid larvae whose adult females are known to benefit from nectar and pollen (Telenga, 1958; Haslett, 1989). Unfortunately, apple orchard floors are generally maintained with a mown grass sod and strips of bare ground beneath the trees, providing few or no floral resources for resident predators (Horton et al., 2003). Thus, the addition of flowers might greatly enhance resource availability for woolly apple aphid natural enemies, perhaps increasing their numbers and improving biological control. Compared to annual crops, fruit orchards are particularly amenable to natural enemy conservation using flowers because of their semi-permanent features and relatively high levels of structural complexity (Simon et al., 2010). The main objectives of this study were to: (1) identify an annual flowering plant highly attractive to natural enemies of aphids; (2) determine whether providing these flowers bolsters enemy densities and improves aphid suppression; and (3) verify that natural enemies readily move from flowers to surrounding apple trees.

## 2. Materials and methods

### 2.1. Screening of flowering plants

Several factors focused our search for flowering plants that would be most useful in our study system. First, the literature reports several flowering plants known to be attractive to adult syrphid flies, which have been reported to be important natural enemies of aphids in the northwestern USA (and elsewhere) that are responsive to the addition of floral resources (e.g., Hogg et al., 2011). Second, we focused on flowering annual plants. This was for purely pragmatic reasons, as in our experience apple growers are more receptive to short-term plantings that can be periodically tilled-under and replanted to avoid becoming weedy (E.H. Beers, personal communication). A literature search led us to the following six plants that met our selection criteria: marigold, *Calendula officinalis*; buckwheat, *Fagopyrum esculentum*; cosmos, *Cosmos sulphureus*; mustard, *Brassica juncea*; zinnia, *Zinnia hybrida*; and sweet alyssum, *Lobularia maritima* (Kloen and Altieri, 1990; Lovei et al., 1993; Ambrosino et al., 2006; Sadeghi, 2008). Plots of these flowers were established in a field area near tree-fruit orchards, and we then monitored flower visitation by adult syrphids, as described below.

Our screening of annual flowering plants was conducted in a field at Washington State University's Tree Fruit Research & Extension Center in Wenatchee, WA, USA. This site was surrounded by apple orchards to the north and west, a cherry (*Prunus avium*) orchard to the south, and buildings to the east; a large tract of unmanaged ground with native shrub-steppe plants (primarily *Artemisia tridentata*, *Purshia tridentata*, *Erigeron* spp., and *Agropyron spicatum*) adjoined the apple orchard on the west. Flowering plants were grown from seed in a greenhouse ( $25 \pm 2$  °C, R.H.  $65 \pm 5\%$ ) in 0.25 m pulp pots filled with potting soil (Miracle-Gro Promixing soil, Marysville, OH) and watered as needed. Ten days after germination, plants were transported to the field site, on 12 August 2008. We established four replicate plots of each of the six flower species, arranged within a completely randomized design. Each plot consisted of 30 pots of that plants species, arranged in three rows of 10 pots, covering roughly  $1 \times 3$  m of ground. Plots were 10.5 m (east-west) or 15 m (north-south) apart, and the existing in-ground irrigation system (impact sprinklers on 0.60 m risers) was used to provide water to the potted plants, which were irrigated twice per week.

Flower attractiveness was measured by recording the flower-visit frequency of adult syrphids during a 2 min observation period per replicate plot, with observations made between 10:00 am and 12:00 pm, on 1, 6, 13, 20 and 27 September 2008. After the 2 min observation period in each plot, we captured adult syrphids using an aerial net; these adults were killed and pinned for later species identification. The temperature for those time slots varied from 23 to 28 °C, and the conditions were sunny for the first, third, and fourth sample dates, and partly cloudy for the second and fifth sample dates.

### 2.2. Effect of sweet alyssum on woolly apple aphid suppression

Having identified sweet alyssum as the most attractive annual flowering plant in objective 1 (see above; Fig. 1), our second goal was to determine whether these flowers significantly increased natural enemy densities and improved woolly apple aphid suppression. Our experimental design included just two treatments: (1) sweet alyssum flowers planted nearby to focal apple trees infested with woolly apple aphid, and (2) mowed grass but no flowers provided near aphid-infested apple trees. The experiment was conducted twice, first in September 2010 and again in October

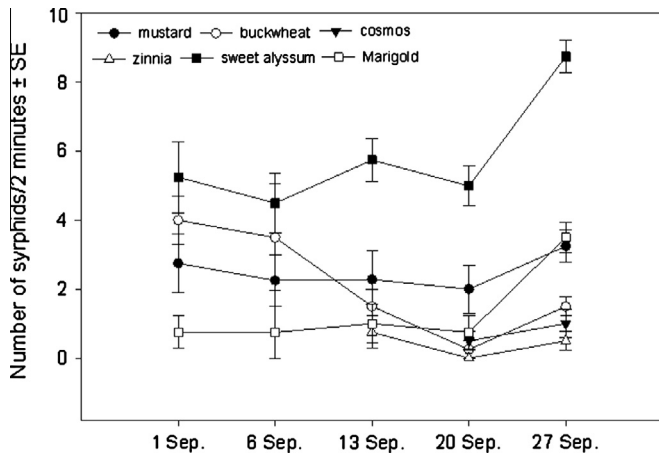


Fig. 1. Mean number of adult syrphids ( $\pm$ SE) attracted to different flowering-plant species during a series of visual counts.

2010, with methodology nearly identical between the two runs (detailed below). There were three replicates of each treatment within each experimental run, arranged within a fully-randomized design, for a total of six plots in each experiment. Each replicate plot ( $3.6 \times 15$  m) consisted of four, 1.2 m-tall potted apple trees that were infested with woolly apple aphid, with two trees in each replicate in each tree row near the plot's north-south center. Potted trees within each replicate plot were 5 m apart, while replicate plots were spaced 120 m apart (north-south, down rows) and 40 m apart (east-west, across rows). Both experimental runs were conducted in a 4-year-old unsprayed WSU research orchard (mix of the cultivars 'Gala', 'Granny Smith', 'Golden Delicious', and 'Jonagold'), bordered by pears (*Pyrus communis*) to the north, apples to the west, cherries (*P. avium*) to the south and a basalt cliff with a narrow strip of native habitat on the east. The orchard floor was covered by short-mown grass between tree rows. Irrigation was provided with a system of micro sprinklers running along the tree rows (south-north). The orchard was watered 2–3 times a week.

The plots randomly assigned to receive the sweet alyssum treatment were tilled with a tractor-drawn rotovator 2 weeks before sowing sweet alyssum seeds (American Meadows, Williston, VT). The seeds were sown by hand at a rate of ca. 150 g of seed per plot. After sowing, the top 1–2 cm of soil was carefully turned over using a rake. Sweet alyssum plots were kept free of weeds prior to the beginning of the experiment by weekly manual removal. Sweet alyssum germination was lower on one plot, which had unusually sandy soil, during the first experimental run; that plot required two additional seeding (the last additional seeding was three weeks before the experiment). A data logger (Hobo U12, Onset Computer Corporation, Pocasset, MA) was installed in the middle of the orchard to monitor temperature.

About four months prior to the experiment, dormant apple trees ('Auvil Early Fuji cultivar 216'/M-9 RN-29; Van Well Nursery Inc. Wenatchee, WA, USA) were transplanted into 0.20 m-pots using the same soil media and in the same greenhouse described above, and allowed to leaf out. A month before the beginning of the experiment each tree was infested with a 2 cm apple twig containing 50–70 woolly apple aphids, with the aphids obtained from a greenhouse colony. Two days before placing the infested trees in the field, the number and size of woolly apple aphid colonies were adjusted such that each tree housed two aphid colonies each covering a  $1 \text{ cm}^2$  area. Aphid colony reduction was achieved using a brush to remove unwanted aphids. This resulted in approximately 1000 aphids (all instars) per tree (about 500 aphids per colony). This aphid density was used in a classic study of woolly apple aphid

parasitism (Mueller et al., 1992), and would be typical of a low-intensity aphid outbreak (Beers et al., 2010). In the first experimental run, potted trees were deployed on 6 September, and data on woolly apple aphid and its natural enemies were collected weekly for three weeks thereafter. In the second experimental run, potted trees were deployed on 27 September and the aphid and natural enemy counts were collected weekly for four weeks thereafter. Altogether, then, the two blocks of the experiment covered much of the typical fall density peak for woolly apple aphid in Washington.

Colony size was used to estimate woolly apple aphid densities based on an equation obtained from a regression between colony surface area and aphid numbers ( $Y = 110.27 \cdot X + 276.11$ ) (Appendix Fig. 1). Estimation of aphid density per tree was done by placing the surface area covered by each aphid colony 'X' into the equation listed above. We used a variety of methods to census predator and parasitoid populations both on and near the focal infested apple trees. First, we conducted a non-destructive visual examination of syrphid activity by visually scanning the orchard floor for adult syrphids hovering over the grass or sweet alyssum, recording the number of adults observed during a two-min period. A small subsample (one or two adult syrphids and/or lacewings) was netted to have their gut contents searched for sweet alyssum pollen. The sub-sampled insects were preserved in 70% alcohol before being dissected the same day. In our dissections the gut content of each individual insect was placed on a glass slide, stained with safranin (Wratten et al., 1995), and examined under a compound microscope at  $4 \times 100$  to determine the presence of sweet alyssum pollen. In addition, a more substantial sample of adult syrphids was done at the end of the second experimental run using an aerial net (adults hovering over the sweet alyssum flowers were collected during one-h period, killed and pinned for later species identification). Next, grass and sweet alyssum plots were sampled with a sweep net (three sweeps/plot), and the natural enemies collected were recorded and released. In addition, natural enemies were sampled on four of the field-planted apple trees bordering the plots with a beating tray (one tap per each of the four trees). Finally, earwigs were sampled on focal trees using a 10 cm roll of cardboard tied to the tree trunk 10 cm above the soil level, as these opportunistic predators are not effectively sample using other means (Horton et al., 2002). The cardboard traps were collected and replaced on each sample date.

### 2.3. Movement of natural enemies between sweet alyssum and apple trees

Our next goal was to verify that predators and parasitoids regularly move from sweet alyssum flowers to adjacent potted focal apple trees, and into the orchard beyond our experimental plots. To accomplish this we sprayed sweet alyssum flowers with egg white, later collected predators at varying distance from the flowers, and used ELISA to test for the presence of egg white protein. This trial was conducted from 27 June to 30 August 2011, in the same orchard where the aphid suppression study was conducted (see Objective 2.2 above). Sweet alyssum seeds were sown, and potted apple trees were infested with aphids, as described above. We planted sweet alyssum into three plots measuring  $15 \times 3.6$  m each, and spaced 40 m apart (east-west, across tree rows). Four potted trees were placed surrounding each sweet alyssum plot, two trees 5 m apart to the north and two 5 m apart to the south. The trees used were heavily infested, housing at least 6000 aphids each. This aphid density would not be unusual during a relatively severe aphid outbreak (Beers et al., 2010), and would be likely to catch the attention of a grower and trigger an insecticide application. In contrast, trees in the surrounding orchard were not infested with woolly apple aphid at the time of this experiment;

thus, the infested potted trees were the only nearby source of this aphid species as prey for the predators. Plots were irrigated 1–2 times a week via a micro-sprinkler irrigation system (after natural enemy collection).

Chicken-egg protein (liquid egg white; All Whites®, Crystal Farms, Minnetonka, MN) was used as a marker to assess the movement of predators and parasitoids between sweet alyssum and apple trees. Sweet alyssum plots were sprayed with a 20% solution of egg whites diluted in water. Water softener (tetrasodium ethylenediaminetetraacetic [EDTA]; The Herbarie at Stone Hill Farm Inc., Prosperity, SC) at 16 g per 10 L of solution was also added to reduce water hardness. The egg white plus EDTA solution was applied once a week using a 16 L backpack sprayer (Smith Sprayers®, Utica, NY). Immediately after each spray, white and yellow sticky traps were hung at a height of 1–1.5 m in the canopies of infested potted trees and uninfested field-grown trees, at the distances described below. Each side of the sweet alyssum plots had a 2 × 2 permutation of sticky trap and tree type (yellow and white sticky traps on both infested potted trees and uninfested field-grown trees). One cardboard band (Horton et al., 2002) was also placed at the base of an infested potted tree and uninfested field-grown tree (one on each side of the alyssum plot). In addition, two white sticky traps parallel to each other and 10 m apart were hung individually in the canopy of field-grown trees as described above at different distances away from the experimental area in the four cardinal directions (50 m on east, 100 and 200 m on west, 100 m on south, and 100 and 200 m on north). Natural enemies were collected 24 h after each spray and trap deployment. Natural enemies were collected from the deployed sticky traps, and at the same time directly from tree canopies (two infested and two uninfested trees from each plot) and sweet alyssum flowers by shaking their limbs and flowers respectively, onto a tray covered by waxed paper coated with adhesive (Tanglefoot®, Grand Rapids, MI).

All specimens caught on sticky traps and directly from tree canopies were processed before those predators collected directly out of sweet alyssum, to reduce the risk of predators becoming inadvertently contaminated with egg white that had been sprayed onto the alyssum flowers. Samples of sweet alyssum flowers and leaves were also collected at each time to confirm the presence of egg protein where it had been sprayed. Natural enemies caught on the sticky traps and tray were removed with the aid of toothpicks (toothpicks were discarded after one use to avoid contamination) and individually transferred to 1.5 ml microtubes. The microtubes were immediately placed into an ice chest for transport to the laboratory. Samples were stored at –4 °C until they were tested for the presence of egg protein. Specimens were removed from storage and washed individually with 100 µl of 1 × PBS (buffer) and then vortexed and centrifuged. The buffer wash was thereafter subjected to indirect ELISA (Jones et al., 2006) for identifying the egg protein marker.

#### 2.4. Data analysis

Our syrphid-visitation data from the initial flower screening, and the aphid and natural enemy density data in aphid-suppression study, each were assessed using repeated measures analyses of variance (PROC MIXED) in SAS v.9.2 (SAS, 2008). Covariance structures for the mixed model repeated measures were constructed, and Bayesian Information Criterion (BIC) were used to assess that the ANTE (1) covariance structure provided the best fit for the flower visitation study, while the CS covariance structure provided the best fit for the aphid suppression study. Date was considered a random factor in these models. For both studies, pairwise treatment comparisons using LS Means (PROC GLM) (SAS, 2008) were conducted separately on each date whenever we observed a statistically significant treatment by date interaction (see

Results; von Ende, 1993). A correlation between the natural enemy and woolly apple aphid density within treatments was also carried out for the experiments described in 2.2 (PROC CORR) (SAS, 2008).

### 3. Results

#### 3.1. Screening of flowering plants

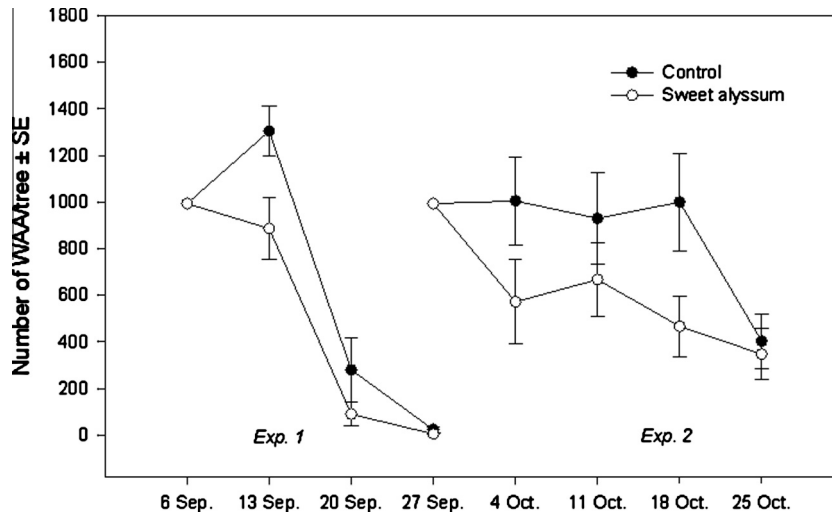
Flower species significantly differed in the number of syrphid adults they attracted ( $F_{5, 90} = 54.40, P < 0.0001$ ), and a treatment by time interaction was also noted ( $F_{20, 90} = 2.62, P = 0.023$ ). The number of syrphids observed in the 2 min observation periods was highest in the sweet alyssum plots throughout the experiment (Fig. 1). The tallest plants were mustard and buckwheat, whereas sweet alyssum was the shortest (Appendix Table 1).

A total of 52 adult syrphids were captured, killed and mounted for identification. Most of the species identified were the pollinators *Eristalis arbustorum* L. ( $n = 41$ ), *Sphaerophoria philanthus* Meigen ( $n = 1$ ), *Parasyrphus relictus* Zetterstedt ( $n = 1$ ), *Eristalis hirta* Loew ( $n = 2$ ), and *Syrpitta pipiens* L. ( $n = 1$ ) which were found feeding on all tested plants with the exception of zinnia. Predatory syrphids were only present among the specimens collected from sweet alyssum and mustard. The predatory syrphid *Eupeodes americanus* Wiedemann ( $n = 2$ ) was observed on sweet alyssum and *Scaeva pyrastris* L. ( $n = 1$ ) on mustard, whereas *Syrphus opinator* Osten-Sacken was observed on both (sweet alyssum,  $n = 2$ ; mustard,  $n = 1$ ).

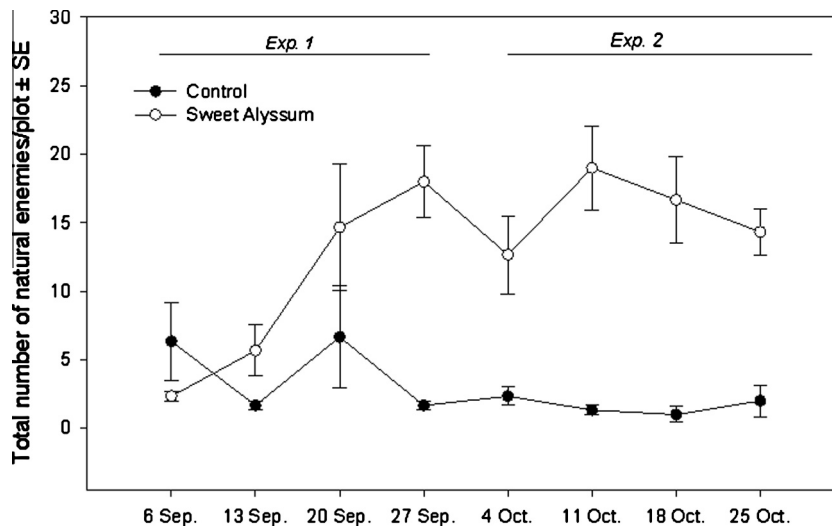
#### 3.2. Effect of sweet alyssum on woolly apple aphid suppression

Results were broadly consistent between the two runs of our experiment. In both runs, aphid densities were significantly lower on apple trees placed next to alyssum flowers compared to controls (treatment main effect; run 1:  $F_{1, 12} = 17.01, P = 0.05$ ; run 2:  $F_{1, 16} = 44.79, P = 0.02$ ; Fig. 2). Aphid densities varied from one date to another within both experiments (time effect; run 1:  $F_{2, 12} = 40.83, P < 0.001$ ; run 2:  $F_{3, 16} = 11.20, P < 0.001$ ; Fig. 2), but suppression of aphids near sweet alyssum plots was consistently greater across dates (treatment × time interaction; run 1:  $F_{2, 12} = 1.21, P = 0.35$ ; run 2:  $F_{3, 16} = 3.03, P = 0.07$ ; Fig. 2). Aphid suppression was mostly due to predation because the parasitism rate by *A. mali* was negligible. In experiment 1, parasitism was zero on all dates. In experiment 2, parasitism was zero on the first two dates and below 1% on the last three dates; parasitism was consistently low in both control and sweet alyssum plots.

Overall, a higher number of natural enemies (summing across all sampling methods) occurred in plots planted with sweet alyssum compared to controls (treatment main effect;  $F_{1, 24} = 126.01, P = 0.0004$ ; Fig. 3; Appendix Table 2), but there was neither a significant date ( $F_{5, 24} = 0.59, P < 0.632$ ) nor treatment by date interaction ( $F_{5, 24} = 1.23, P = 0.34$ ). In general, more ambient natural enemies were found in the ground cover (sweep nets) than on field-planted apple trees (beating trays) (Appendix Table 2). Spiders were the most abundant predator found in either sample type. Spiders were not identified to species, but other researchers report a tremendous diversity of spiders in Washington apple orchards (Horton et al., 2012). Lacewing adults were moderately abundant in tray samples, but not in sweep nets. Adults of the aphid parasitoid *A. mali* were relatively rare in both sample types. Earwigs were moderately abundant in cardboard bands during run 1 (September), but almost absent during run 2 (October), likely reflecting their movement to overwintering shelters as winter approached (Horton et al., 2002). Relatively few natural enemies were collected on potted apple trees (Appendix Table 3).



**Fig. 2.** For two field experiments, the mean number of woolly apple aphids ( $\pm$ SE) on apple trees placed near plantings of alyssum flowers (sweet alyssum) or near short-mown grass with no flowers present (control). The last sampling date of the first experiment (exp. 1) coincided with the deployment of infested trees in the second experiment (exp. 2).



**Fig. 3.** For two field experiments, the mean number of natural enemies ( $\pm$ SE) on or nearby the focal apple trees placed near plantings of alyssum flowers (sweet alyssum) or near short-mown grass with no flowers present (control). The last sampling date of the first experiment (exp. 1) coincided with the deployment of infested trees in the second experiment (exp. 2). Natural enemies were summed across several sampling methods (described in text) and taxa (Table 1). Natural enemies include: syrphids, lacewings, coccinellids, earwigs, damselbugs, spiders, mirids, parasitoids (*A. mali*).

In a quantitative sample in both treatments, adult syrphids were found exclusively in the sweet alyssum plots, with a peak of approximately 12 specimens per 2 min observation (Appendix Table 2). In a qualitative sample, seven species of adult syrphid were collected in or near the sweet alyssum plots (Appendix Table 4). Syrphid species that were collected included *Syrphus opinautor* Osten-Sacken (35), *Eupeodes fumipennis* Thomson (7), *Scaeva pyrastris* L. (3), *Eupeodes volucris* Osten-Sacken (2), *Syrphus ribesii* L. (2), *E. americanus* Wiedeman (1), and unidentified Eristalinae (pollinator) (1). Of the predatory species found, all except *S. pyrastris* and *E. volucris* have been observed to feed on woolly apple aphid colonies in previous studies (Walker, 1985; Gontijo et al., 2012), and the latter two are known predators of green apple aphid *Aphis pomi* (Carroll and Hoyt, 1984).

Six out of eight predatory adult syrphids collected throughout the experiments were identified as positive for sweet alyssum pollen in their gut, confirming that the syrphids were using these flowers as a food source (Appendix Table 4). Although lacewings

are known pollen feeders (Venzon et al., 2006), all the eight adults collected at the same time as the syrphids tested negative for sweet alyssum pollen.

### 3.3. Movement of natural enemies between sweet alyssum and apple trees

The most-commonly collected natural enemies were spiders, anthocorids, syrphids, chrysopids, coccinellids, earwigs, and the parasitoid *A. mali*. Among these, 33–100% of individual predators found on the infested trees carried sufficient egg-white protein to be scored as positive for having visiting our marked sweet alyssum flowers (Table 1; data are summed across both white and yellow traps). The predatory mirid *Deraeocoris brevis* was the only predator found on the trees that did not test positive for the marker; however, it was caught in very low numbers. Nabids and geocorids were the only predators that were only caught on sweet alyssum flowers (Table 1). Ca. 40–50% of the syrphids caught

**Table 1**

Percentage of natural enemies from sweet alyssum, apple trees and distant traps that tested positive for the protein marker.

Natural enemies	S. alyssum		Infested trees		Uninfested		50 m <sup>a</sup>		100 m <sup>a</sup>		200 m <sup>a</sup>		Total	
	N	% Positive	N	% Positive	N	% Positive	N	% Positive	N	% Positive	N	% Positive	N	% Positive
Syrphidae	1	100.0	19	47.4	23	30.4	0	0.0	8	50.0	5	40.0	56	41.1
Chrysopidae	0	0.0	59	42.4	16	62.5	0	0.0	3	0.0	1	0.0	79	44.3
Chrysopidae larvae	0	0.0	8	50.0	0	0.0	0	0.0	0	0.0	0	0.0	8	50.0
Coccinellidae	0	0.0	9	33.3	3	33.3	0	0.0	1	0.0	0	0.0	13	28.6
Coccinellidae larvae	0	0.0	2	100.0	0	0.0	0	0.0	0	0.0	0	0.0	2	100.0
<i>Deraeocoris</i> spp.	0	0.0	2	0.0	1	0.0	0	0.0	0	0.0	0	0.0	3	0.0
<i>Nabis</i> spp.	11	81.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	11	81.8
Anthocoridae	9	77.7	2	50.0	0	0.0	0	0.0	0	0.0	0	0.0	11	72.7
Geocoridae	3	33.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	33.3
Forficulidae	0	0.0	7	42.8	18	33.3	0	0.0	0	0.0	0	0.0	25	36.0
Spiders	20	95.0	4	50.0	4	75.0	0	0.0	0	0.0	0	0.0	28	85.7
<i>Aphelinus mali</i>	0	0.0	26	50.0	17	70.5	2	50.0	1	100.0	0	0.0	46	58.7
Total	44	84.1	138	47.32	82	47.6	2	50.0	13	38.5	6	33.3	285	50.9

<sup>a</sup> White sticky traps at different distances away from experimental area.

on sticky traps 100–200 m from the marked flowers were also marked, indicating this species can disperse considerable distances in short periods.

#### 4. Discussion

The addition of flowering plants to agroecosystems is a conceptually simple means to increase densities of predators and parasitoids, because so many natural enemies can use pollen and/or nectar as food (Landis et al., 2000). Nonetheless, this approach often fails to improve pest suppression (e.g., Bugg and Dutcher, 1993; Bone et al., 2009). We examined the use of the flowering annual plant sweet alyssum to foster higher densities of natural enemies of the woolly apple aphid in Washington (USA) apple orchards. Our results provide initial evidence that this approach could be effective. In two successive field experiments we observed a rapid, sustained decrease in woolly apple aphid densities on trees placed next to sweet alyssum plantings (Fig. 2). Falling aphid densities were associated with a dramatic increase in natural enemy populations within and near sweet alyssum, consisting primarily of a diverse group of generalist predators known (or likely) to attack woolly apple aphid (Fig. 3, Appendix Table 2). Using egg-white protein as a marker, we were able to document movement of these natural enemies not only onto adjacent aphid-infested trees, but in some cases hundreds of meters into the surrounding orchard (Table 1). Thus, we suggest that predators and parasitoids drawn into sweet alyssum to feed on nectar and/or on pollen, readily moved out onto surrounding apple trees where they had the opportunity to attack aphids. Nonetheless, natural enemy densities quickly dropped off with increasing distance from the flowering pots (Table 1).

Previous work in our region, and elsewhere, has suggested that syrphid flies are particularly important aphid natural enemies and ones that gain from the addition of flowering plants to an agroecosystem (Colley and Luna, 2000; Sutherland et al., 2001; Hogg et al., 2010). Thus, we initially focused our attention on syrphids, which had appeared to have the potential to be important woolly apple aphid predators in Washington apple orchards (Gontijo et al., 2012). We observed that sweet alyssum was attractive to syrphids in our apple-orchard system (Fig. 1). The low-growing habit of sweet alyssum and its ability to be quickly and easily re-seeded, match well with local tree fruit growers' desire for a flowering plant that interferes minimally with standard orchard-management practices. Despite its clear attractiveness to syrphids, however, several lines of evidence suggest that natural enemies other than syrphids were responsible for the aphid suppression that we observed. First, it is the syrphid larvae that kill aphids, and

the process of adult flies locating aphid infestations and ovipositing within them, fly eggs developing, and larvae reaching the larger and relatively voracious later stages likely would require several weeks before yielding a heavy impact on aphids (Bergh and Short, 2008). In contrast, we saw a substantial reduction in aphid densities near sweet alyssum in just seven days. Second and most convincingly, syrphid larvae were rarely collected on or near our aphid-infested apple trees (Appendix Table 3). Syrphid larvae typically stay within the aphid colonies where they are feeding (Schneider, 1969), and thus would be expected to commonly be nearby or within aphid colonies. Rather, we found a diverse community of spiders and predatory bugs associated with sweet alyssum plantings (Appendix Table 2), suggesting that these natural enemies may have been partly or largely responsible for aphid suppression near flowers. Spiders and predatory bugs often are broad generalists that forage widely and often at night, which could explain why we rarely saw these predators on the trees themselves during our diurnal collections and observations. Similarly, earwigs are efficient nocturnal predators of woolly apple aphid (Asante, 1997) and have been associated with successful biological control of woolly apple aphid in several studies (Mueller et al., 1988; Ravensburg, 1981).

Although aphid densities were similar in sweet alyssum and grass plots at the end of both experiments, a faster aphid reduction was consistently observed in the first week in the sweet alyssum plots compared to controls (Fig. 2). In experiment 1, woolly apple aphid numbers were reduced to nearly zero in four weeks on both treatments. In experiment 2, aphids were reduced to about half of the initial numbers over 5 weeks (Fig. 2). The slower aphid reduction observed in experiment 2 may be due to the lower temperatures prevailing in October (20/10 °C max/min) versus September (25/14 °C max/min), which may have reduced predator activity. Indeed, on the infested potted trees, more natural enemies were found in experiment 1 (control,  $n = 102$ ; sweet alyssum,  $n = 41$ ) than in experiment 2 (control,  $n = 6$ ; sweet alyssum,  $n = 0$ ) (Appendix Table 3). This may help explain why late-season outbreaks of woolly apple aphids are common in Washington (Beers et al., 2010); woolly apple aphids have a relatively low optimal developmental temperature and thus can maintain a high rate of reproduction during cool temperatures when predators are less active (Asante et al., 1991).

Although our results are encouraging, our study has some obvious limitations. The aphid-suppression experiments in total covered just two months, albeit encompassing the time of the year when woolly apple aphid problems are usually most severe. Thus it remains unclear whether alyssum plantings could encourage a season-long improvement in aphid suppression. Likewise, we

measured pest control only on trees immediately adjacent to the flowers, and not throughout the orchard. The limitations of conservation biological control schemes often are revealed only when experimenters consider a longer time period or broader physical scale, or when more species are examined than a single pest and its natural enemies (Marino and Landis, 1996; Landis et al., 2000; Frank et al., 2008). It was clear that natural enemies did aggregate at alyssum flowers (Fig. 3), and that these same enemies readily moved out into the surrounding orchard beyond our focal potted trees (Table 1). Thus, alyssum flowers may have benefits at scales greater than we examined, although this remains to be tested. Future work should investigate whether parasitoids or intraguild predators of key natural enemies also benefit from alyssum flowers (Araj et al., 2006; Prasad and Snyder, 2006). Sometimes, intraguild predators build-up through time in refuge plantings and cause harm to their predators species one is attempting to conserve (e.g., Jonsson et al., 2009). Likewise, orchard pests such as western flower thrips (*Frankliniella occidentalis*) may also benefit from the presence of a flowering plant, although this potential problem could be managed using minor changes in cultural practices (i.e., re-seeding the alyssum patches after apple bloom, the only time apple fruits are susceptible to thrips damage). Critically, further work is needed to determine whether the costs associated with growing and maintaining alyssum or other flower species is justified based on any savings in chemical pest control. Flowers also must be avoided during tractor or other field work, an additional possible complication. In the work presented here we examined only single species of flowers, whereas reliability in flower establishment and/or an extension of total flowering time might be achieved through multi-species flower mixes. This possibility has yet to be examined for our system, although of course with more flower species comes a heightened risk of unintended use of some resources by pests or intraguild predators as described above. Nonetheless, increasing biodiversity can bring great and unexpected benefits for biological control (Straub et al., 2008).

In summary, this three-year study suggests the potential for sweet alyssum plantings to enhance biological control of woolly apple aphid. Insectary plantings are a severely underutilized tactic in western USA orchards, and a system that is both compatible with orchard management and effective against a difficult target pest deserves wider implementation. This is especially true for organic farmers who have very few insecticide options available and need to use preventative, rather than remedial, measures. Future work should consider a broader spatial, temporal, and ecological context, to make certain that conservation biological control using sweet alyssum would be both economically feasible and consistently effective.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocontrol.2013.03.007>.

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