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"Attract and reward" for syrphid flies using methyl salicylate and sweet alyssum in kale in north Florida

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ABSTRACT

"Attract and reward" is an ecologically-based pest management technique for improving biological control. A predator attractant, such as an herbivore-induced plant volatile (HIPV) is used to "attract" the biological control agent. The predator is sustained and nourished by an insectary plant which acts as a "reward". The "attract" and "reward" effects are expected to act synergistically in enhancing the effectiveness of the predator. Here we tested methyl salicylate to attract syrphid flies, and sweet alyssum (*Lobularia maritima* L. Desv.) to reward them in a kale (*Brassica rapa* L.) field in north Florida. In spring 2014, sweet alyssum in kale showed higher incidence of syrphid fly visitations compared to untreated control fields. The most abundant syrphid fly species was identified as *Toxomerus marginatus* (Say). The Fall 2014 experiment compared syrphid fly counts using MeSA, sweet alyssum, MeSA + sweet alyssum, and an untreated control. Analysis was possible only for *T. marginatus*, although several other species of syrphids were found and recorded. Sweet alyssum was found to increase incidence of *T. marginatus.* However, the addition of MeSA did not increase syrphid fly counts in either the sweet alyssum or untreated controls. The pattern was repeated in spring 2015, but did not attain statistical significance. In attract and reward for syrphid flies in kale, sweet alyssum has shown potential as a reward component, but MeSA proved ineffective as an attractant. Other HIPVs should be evaluated to develop an effective attract and reward system for syrphid flies.

Additional index words: *Brassica rapa*, *Lobularia maritima*, generalist insect predator, *Toxomerus marginatus*

A common technique in ecologically-based, holistic pest management is the use of insectary plants that provide nectar or pollen for beneficial insects, such as syrphids. Syrphid flies (Diptera: Syrphidae) are important pollinators and generalist predators in various agroecosystems and are known to benefit from the presence of insectary plants such as sweet alyssum, *Lobularia maritima* L. Desv. (Bassicales: Brassicaceae) (Pinheiro et al. 2015). Methyl salicylate (MeSA) is an organic compound produced by many plant species, particulary wintergreens. MeSA is an "herbivore-induced plant volatile" (HIPV), and is known to attract natural enemies (Rodriguez-Saona et al. 2011). Meta-analysis of published experiments has shown strong evidence that insect predators are broadly attracted to MeSA in agricultural systems, although research is needed into the extent to which synthetic MeSA may be used to improve biological pest control.

"Attract and reward" is a pest control strategy combining the effects of a synthetic HIPV, such as MeSA (to "attract"), with an insectary plant expected to sustain ("reward") the predator populations (Simpson et al. 2011a, Gordon et al. 2013). The combined approach is intended to yield synergistic effects to improve biological control. In this study, we investigated the use of MeSA (PredaLure ™) to "attract" syrphids, with sweet alyssum as "reward" in kale in north Florida.

MATERIALS AND METHODS

Effect of sweet alyssum alone. *(Spring 2014, April 2 – June 3, 2014)* The relative visitation of different species of syrphid flies was measured using sweet alyssum plants, *Lobularia maritima* (L.) Desv. (Brassicales: Brassicaceae) (var. "Carpet of Snow") as an insectary plant in small kale, *Brassica rapa* L. (Brassicaceae) (var. "Siberian Dwarf") in plots in North Florida. The kale host plants were seeded in the greenhouse on 17 January 2014 using Sunshine LT5 tobacco mix® (Sun Gro Horticulture, Agawam, MA). Kale was transplanted to the field after danger of frost had passed. Sweet alyssum was seeded weekly in the greenhouse (beginning 8 Jan 2014) to ensure that a steady supply of inflorescences was available. Sweet alyssum was then transplanted (about 2-3 month old seedlings) to 13 gal (≈50 l) pots and was taken to the field as needed. The experimental area was located at the USDA-ARS-CMAVE in Tallahassee, Florida. Four plots were established that consisted of four 30 m long beds of black plastic. The beds were on 1.8 m centers, planted in double rows. Kale plants were spaced 0.3 m apart. Two plots were located over 200 m away from the other plots. The other two plots were located 74 m apart. The experimental area was bordered by a residential subdivision to the east and south. Between the plots were soybean and lupin fields, bahia pasture, muscadine grapes and mixed vegetable gardens.

Insect sampling was performed using Malaise traps for reasons described elsewhere, especially because of sensory neutrality and lack of insect specificity (Miller et al. 2013). Malaise traps (BioQuip Products Inc. Rancho Dominguez, CA, model 2875D) were set up in the middle of each plot at the end of March, at the time of transplanting. Sweet alyssum treatments were randomly assigned to two of the four plots at the start of the sampling period. In sweet alyssum plots, ten pots of sweet alyssum were placed in the center of the plot under and around a single malaise trap. The two plots that did not receive sweet alyssum served as control plots. All four plots were sampled for syrphid flies using malaise traps for one 24-hour period each week.

Sweet alyssum in combination with PredaLure ™ (methyl salicylate, MeSA). The effects of sweet alyssum in combination with PredaLure (Agbio, Westminster, CO) were studied over two seasons: Fall 2014 and Spring 2015 at the USDA-Agricultural Research Service, Center for Biological Control in Tallahassee, FL. Syrphid flies were captured using malaise traps based on Townes (1972) as described above. These consisted of a horizontal mesh barrier "wall" held in place by 2 aluminum poles and with shorter mesh perpendicular-extensions at both ends. There was also a mesh sloping roof that ran along both sides of the central-wall. Traps were 1.8 meters long by 1.2 meters wide and had an opaque plastic collecting jar located at the top of one pole. Ethanol (95%) was added to a depth of 2-3 cm to preserve the trapped insects.

Fall 2014 (October 29 to December 8 2014) and Spring 2015 (April 28 to May 20, 2015). Malaise traps were placed on top of a 6 m^2 area covered with black weed block. Four traps were grouped in each of two locations. Traps were placed in a line 30 m apart on the edge of a muscadine vineyard with a mixed grass and forb ground cover at the first location. At the second location traps were arranged 30 m apart in each corner of a square near a chestnut orchard. Each trap at both locations was assigned one of the following treatments: 1) MeSA (PredaLure) 2) Eight potted sweet alyssum plants (var. "Snow Princess") 3) MeSA + eight pots of sweet alyssum and 4) Control (empty trap). Predalure packets were attached with a safety pin to the middle of the center wall of the trap, approximately 20 cm lower than the upper mesh canopy. Trap heads were placed on traps and insects were captured for 48-hour periods. Methyl salicylate treatments were 5 gram slow release PredaLure packets. New MeSA packets were used for each replicate test. At the start of each trapping period treatments were rotated to account for position effects.

Statistical Analysis. Comparison between adult mean syrphid counts in plots with sweet alyssum versus those without sweet alyssum was performed using a paired *t*-test. Effects of sweet alyssum in combination with MeSA was analyzed using One-Way Analysis of Variance. All analyses were performed using SYSTAT (Systat Software, San Jose, CA).

RESULTS

Effect of sweet alyssum alone. The vast majority of syrphids captured were *Toxomerus marginatus* (Say). In increasing order, the total numbers of syrphid flies captured during the sampling period were: *Pseudodoros clavata* F. (2); *Syritta pipiens* (L.) (2); *Eristalis* spp. (2), *Chalcosyrphus* spp. (2), *Toxomerus boscii* Macquart (3); *Toxomerus geminatus* (Say) (5), *Ocyptamus fuscipennis* (Say) (8), *Eupeodes americanus* Wiedemann (13), *Allograpta obliqua* (Say) (19), and *Toxomerus marginatus* (Say) (446).

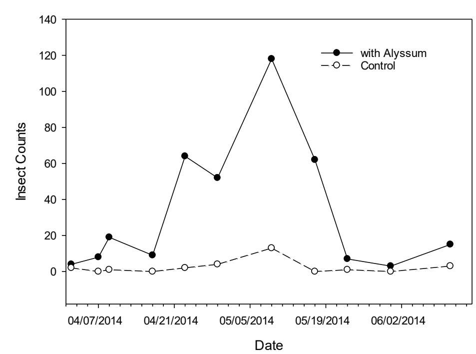

Fig. 1. Total counts of *Toxomerus marginatus* sampled by malaise traps in kale, with and without sweet alyssum treatments during spring 2014 sampling season.

The numbers of *Toxomerus marginatus* captured in kale plots with sweet alyssum were significantly higher than those without sweet alyssum (Fig. 1) (mean ± SE with sweet alyssum: 32.82 ± 11.1; without sweet

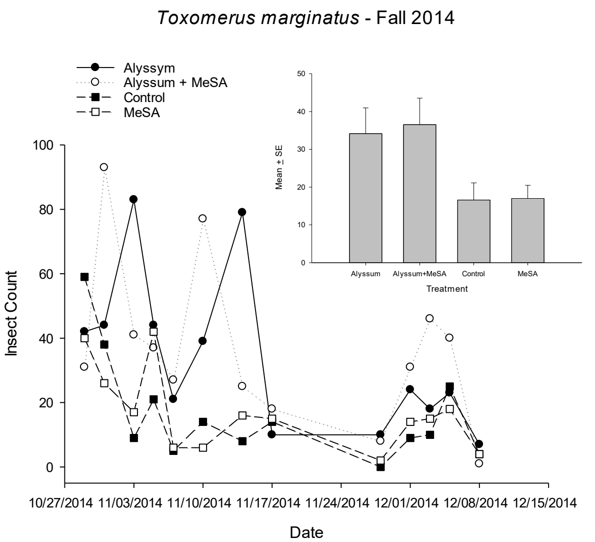

Fig. 2. *Toxomerus marginatus* (mean ± SE) during fall 2014 sampling season. Treatments consisted of sweet alyssum, methyl salicylate (MeSA), sweet alyssum + MeSA, untreated control. Inset shows overall mean for the season (± SE) for each treatment (ANOVA: $F = 3.6$, $R^2 = 0.18$, $P = 0.02$).

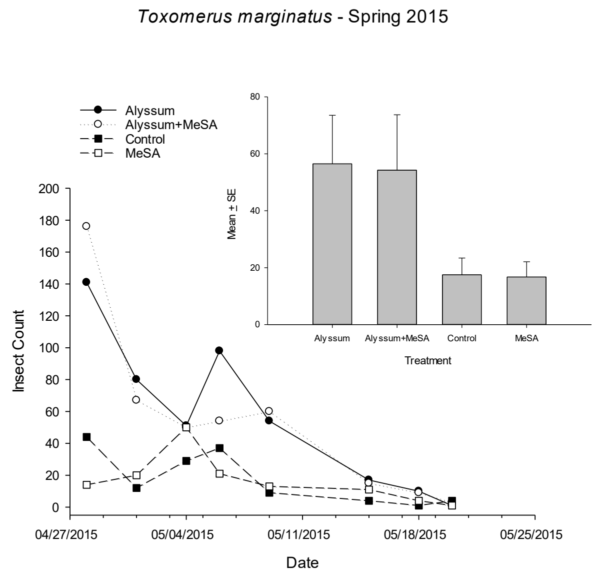

Fig. 3. *Toxomerus marginatus* (mean ± SE) during spring 2015 sampling season. Treatments consisted of sweet alyssum, methyl salicylate (MeSA), sweet alyssum + MeSA, untreated control. Inset shows overall mean for the season (± SE) for each treatment (ANOVA: $F = 2.7$, $R^2 = 0.22$, $P = 0.07$).

alyssum: 2.36 ± 1.1; paired *t*-test = 2.73, df = 20, P = 0.012).

Sweet alyssum in combination with MeSA. Numbers of *T. marginatus* sampled in the fall of 2014 were significantly higher in the Malaise traps with sweet alyssum. The addition of MeSA did not result in any significant increase in numbers of syrphids sampled, although the effect of sweet alyssum alone was significant (Fig. 2) (ANOVA: $F = 3.6$, $R^2 = 0.18$, $P = 0.02$). The incidence of syrphid flies with MeSA alone was not significantly different from traps without any reward (control). The same trend was apparent in the spring 2015 season (Fig. 3), but was not significantly different (ANOVA: $F = 2.7$, $R^2 = 0.22$, $P = 0.07$). All species of syrphids sampled during both seasons are shown in Table 1.

Table 1. All syrphid fly species sampled (Fall 2014, Spring 2015).

Syrphid fly species	Fall 2014	Spring 2015
Toxomerus marginatus	1356	1160
Allograpta obliqua	11	11
Eupeodes americanus	14	0
Ocyptamus fuscipennis	18	1
T. geminatu	36	8
T. boscii	4	13
Pseudodoros clavata	2	2
T. floralis	0	14

DISCUSSION

The origins of the "attract and reward" approach can be traced to Orre et al. (2009). Their objective was to combine ecologically-friendly tactics of using synthetic attractants to increase natural enemy populations, with inter-planting floral resource plants. Whereas the typical use of insectary plants may result only in re-distribution of natural enemies, the combined approach sought to extend conservation biological control by first attracting predators from surrounding habitats, then providing food resources to enhance their fitness. The complementary tactics are intended to produce synergistic enhancement of biological control. Their initial evaluations in turnip (*Brassica rapa* subsp. *rapa*) showed MeSA to increase the abundance of natural enemies, but also of other insects. They concluded that the use of attractants may have unintended effects on the entire insect community (Orre et al., 2009).

Several HIPV formulations, including MeSA, methyl jasmonate (MeJA) and methyl anthranilate (MeA) were used as attractants and buckwheat, *Fagopyrum esculentum* Moench (Caryophyllales: Polygonaceae) as the reward in sweetcorn (*Zea mays*), broccoli and wine-grapes (*Vitis vinifera*), with generally positive results (Simpson et al. 2011a). Attract and reward was tested in brassica crops in New Zealand using MeSA to attract and buckwheat to reward (Gordon et al. 2013). The treatments yielded variable results depending on natural enemy sampled, but generally, no synergistic effects were found. MeSA alone or in combination with coriander was found to attract different predators and alter pest communities, but did not result in improved crop productivity (Salamanca et al., 2018). Furthermore, the spatial arrangements of HIPV dispensers and insectary plants can strongly influence foraging behavior and therefore the effectiveness of field implementation (Jaworski et al., 2019). Separate attract and reward tactics may thus enhance natural enemy populations, but producing synergistic effects will require further research (Simpson et al., 2011b)

Our studies show that sweet alyssum attracts several species of syrphid fly, the most abundant by far being *Toxomerus marginatus*. Syrphid flies sampled within the genus were *T. geminatu, T. boscii* and *T. floralis.* Other syrphid fly species found were *Allograpta obliqua, Eupeodes americanus, Ocyptamus fuscipennis,* and *Pseudodoros clavata*. As in previous studies (Simpson et al., 2011a, Gordon et al., 2013; Salamanca et al. 2018), we found no synergistic effects from the combination of sweet alyssum and MeSA. In fact, the addition of MeSA did not increase the numbers of *T. marginatus* flies sampled. The discrepancy between our finding and previous works may be due in part to the attractive effects of sticky traps used in other studies. Further, the addition of MeSA showed no significant effect compared to the untreated control. However, syrphid fly adults were usually significantly higher in kale plots and traps containing sweet alyssum. Although intended as the "reward" component, sweet alyssum also displayed attractive characteristics, likely due to its pollen and nectar. Insect pests in kale, such as diamondback moth and aphids. were observed to be low during most of the season and increased only at the end. Population densities of pests and the syrphids were too variable to analyze through statistical correlation. In conclusion, sweet alyssum attracted syrphids, resulting in higher sampling counts, but the addition of MeSA showed no effects on the untreated control or the sweet alyssum treatment suggesting it is not a suitable "attract" component for syrphid flies in kale. Further research using other HIPVs is warranted.

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