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Evaluating Mustard as a Potential Companion Crop for Collards to Control the Silverleaf Whitefly, *Bemisia argentifolii* (Hemiptera: Aleyrodidae): Olfactometer and Outdoor Experiments

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ABSTRACT

Three varieties of mustard, *Brassica juncea* (L.) Czern. (Giant red, Tender green and Ragged leaf) were evaluated as possible repellent companion crops for collards (*Brassica oleracea* var. *acephala* de Condolle) against the silverleaf whitefly, *Bemisia argentifolii*, through laboratory studies using a Y-tube olfactometer and outdoor potted experiments. The olfactometer studies did not confirm attraction of adult whitefly females towards collards versus a clean air control. Although a higher number of adult females selected the collards versus the clean air control, the result was not statistically significant. Some test insects attempted to retreat from the air sources. Movement 7 cm or more away from the air sources was classified as “repellency”. When provided with a choice between a clean air control and Giant red mustard, two whiteflies selected the mustard, versus 14 that were either repelled or chose the clean air (P = 0.0042). When presented a choice between collard or mustard odors, one female each chose the mustard and collard air sources and 17 were repelled (P < 0.001). Although the olfactometer bioassays showed evidence of repellent effects of mustard volatiles, we discuss difficulties in the use of the Y-tube olfactometers to study insect repellency. The outdoor potted experiments showed significantly higher numbers of whitefly landings on leaves of collards than on any of the mustards. When collards pots were grouped together with mustards as companion crops, whitefly landings were also lower than those on collards presented as monocultures. However, analyses of landings with time showed no initial preference for collards (P = 0.1), nor avoidance of giant red mustard (P = 0.36). Furthermore, analysis of egg counts showed ovipositional preference for collards as compared to any of the mustard varieties. Numbers of eggs laid did not differ significantly among the crop combinations, suggesting that companion cropping with any mustard variety tested conferred no deterrence against oviposition by this whitefly on collards. Future research needs are discussed.

Additional Index Words: intercropping, repellent crops, trap crops, oviposition preference

Increased consumer demand for organically grown crops, together with environmental and economic problems associated with conventional agriculture, have generated considerable recent research in sustainable pest management strategies that incorporate cutting-edge sciences such as landscape and chemical ecology (Zehnder et al. 2007). One such strategy is the deployment of companion crops with repellent or masking volatiles to disrupt insect pest host-finding behavior (Ratnadass et al. 2011).

Experiments to evaluate the effectiveness of repellent or nonhost companion crops have yielded mixed results. Specific instances have shown that intercropping with reputedly repellent crops can result in a range of effects, from reducing pest incidence, to having no effect, or sometimes to both effects. Examples of repellent crop effects include the following: Intercropping cotton (*Gossypium barbadense* L.) with a putative repellent, basil (*Ocimum basilicum* L.), resulting in 50% reduction in the pink bollworm (*Pectinophora gossypiella* Saunders) (Lepidoptera: Gelechiidae) in Egypt (Schader et al.

2005). Reduced attacks by the carrot fly, *Psila rosae* F. (Diptera: Psilidae), in carrots were attributed to volatile deterrents from an onion intercrop (Uvah and Coaker 1984). Similarly, volatiles from molasses grass, *Melinis minutiflora* Beauv. (Poales: Poaceae), acted as oviposition deterrents against the spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), in a maize intercrop (Kimani et al. 2000).

However, not all studies produced clear evidence of repellent effects. Three reputedly effective companion species, rue (*Ruta graveolens* L.), zonal geranium (*Pelargonium* X *hortorum* Bailey), and garlic chives (*Allium scheonparum* L.) were interplanted with roses (*Rosa* X *hybrida* "Ultimate Pink"), to protect the roses against the Japanese beetle (*Popillia japonica* Newman [Coleoptera: Scarabaeidae]). However, comparison against rose-only control plots showed no significant decreases in numbers of Japanese beetles on roses (Held et al. 2003). In the case of tomato intercrops in cabbage against the diamondback moth (*Plutella xylostella* L. [Lepidoptera: Plutellidae]), Buranday and Raros (1973) reported reductions in oviposition and development attributed to the volatile emissions from tomato. In contrast, similar studies on tomato and cabbage, or tomato and *Vicia fava* L. bean intercrops, showed no effects of population levels on the diamondback moth (Chelliah and Srinivasan 1986, Badenes-Perez et al. 2005).

In a recent greenhouse study, the Giant red mustard, *Brassica juncea* (L.) Czern. (Brassicales: Brassicaceae) demonstrated possible repellent effects against the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae) = *Bemisia tabaci* (Gennadius) (Legaspi 2010). Pots planted with pairs of mustard and collard, *Brassica oleracea* L. var. *acephala*, contained fewer whitefly eggs than those planted to collard-only pairs. However, repellency of a companion mustard crop was not shown in a field setting (Legaspi 2010). Here, we tested the possible effects of plant volatiles from collards and three mustard varieties on whitefly movement and oviposition in olfactometer and outdoor experiments.

MATERIALS AND METHODS

Olfactometer experiment A laboratory experiment was conducted on whitefly response to odors of collards and Giant red mustard, which was selected because it showed most potential as a repellent. Female whiteflies, *Bemisia argentifolii*, used in the experiment came from a laboratory colony reared on tomato, var. Florida 47 (Siegers Seed Co., Holland, MI). Experiments were conducted under fluorescent lighting at a temperature of about 25 °C. The olfactometer consisted of two glass odor chambers (41 cm height, 16 cm diam.) connected to separate glass arms of the Y-tube with silicone tubing (0.6 cm o.d.) (Analytical Research Systems, Inc., Gainesville, FL). The olfactometer was marked at 1-cm intervals and calibrated to an air flow of 1.3 LPM through both tubes. The Y-tube was surrounded by 36 cm high walls made from white foam core poster board in order to standardize light around each olfactometer arm.

Whole plants grown in small plastic pots (11 cm height, 11.5 cm diam.) were placed in the odor chambers. Plant treatments consisted of either Giant red mustard or collards that had been grown to a height of about 14 cm. The pots were covered with aluminum foil up to the main stem of each plant to reduce odors coming from potting soil. A pot containing soil covered with foil was used in one odor chamber in tests where response to a whole plant was compared to that of a control. Both odor chambers contained moistened filter paper. In a preliminary test female whiteflies did not respond significantly to odor of collards, a documented host plant, compared to the control. By lacerating the leaves, movement was elicited from the whiteflies after leaves of the plant were cut (1 cm length) several times using a scalpel.

Experiments consisted of comparing whitefly female reaction to pairs of volatile stimuli introduced in each of the 2 arms of the Y-tube. A single adult female whitefly was placed into the open end of the Y-tube at the 0 cm mark using a blackened straw and netting, allowing the insect to move towards the airflow from one of the arms. A movement of 7 cm towards an airflow was designated as a positive response to that treatment, and an insect that moved 7 cm away from an airflow was designated as "repelled" (Dogan and Rossignol 1999). Insects that failed to register sufficient movement after 5 min were categorized as "no decision" and omitted from statistical analysis. Each female was used once and constituted a replicate. After 15 replicates, placement in the tubes was switched and another 15 replicates were performed. These 30 replicates comprised one bioassay; 3 bioassays were conducted. The following pairs of treatments were assayed together:

Bioassay 1. Control pot vs collard plant, var. Georgia
Bioassay 2. Control pot vs Giant red mustard plant
Bioassay 3. Giant red mustard plant vs collard plant

Outdoor potted experiment An experiment on whitefly, *B. argentifolii*, repellency was conducted using potted plants. Testing took place concurrently at 4 different sites on the grounds of the USDA-ARS-CMAVE, Center for Biological Control in Tallahassee, FL. To enhance whitefly response, test plants were presented on 1.2 x 2.4 m sheets of plywood painted a shade of brown with low reflectance. A similar sheet of plywood was placed vertically behind the pots of plants to increase uniformity in treatment background between the sites. All plants were grown in 3 liter plastic pots (height = 19.5cm, diameter = 18.5 cm, and provided with a commercial fertilizer, Miracle-Gro (The Scotts Co., Marysville, OH) once a week. Evaluation was performed on plants that had grown to a height of approximately 23cm tall (~4 weeks old).

The mustard varieties tested included Giant red, Tender green and Ragged leaf mustards. All collards evaluated were of the variety "Georgia". Each of the three sites served as a test plot for one different variety of mustard (collard and mustard combination) and one site was used for the control treatment (collard alone). Sites were randomly assigned to treatments on each day of testing. For each test day, six test plants were placed at each site. Three of these plants were of the same mustard variety while three of the plants were collard controls. These varieties were alternately placed in a line on wooden boards (1.2 x 2.4 m) with pots placed 34 cm apart. A different variety of mustard was used at each of the three sites, and one site had six pots of collards as an additional control. All sites faced south. For sources of infestation, six hundred whiteflies were added to each cage of potted infested tomato plants (var. Florida 47) before each trial. At the start of the experiment, four potted tomato plants infested with whiteflies were removed from the cage and placed on an additional 4x8 sheet of plywood about 2.5 m in front of each site. All tomato plants were put into position at the start of each replicate trial at 0900 h. Subsequently, whiteflies were counted every hour for 6 h. In addition all plants were left exposed to whiteflies for a total of 24 h. On the following day, all leaves were removed from each plant and kept under refrigeration until they could be examined under a dissecting microscope to determine the number of eggs laid on each plant.

Statistical analysis Olfactometer tests were analyzed using 2-tailed binomial tests where the null hypothesis assumed $P = 0.5$ for entry into either tube (Zar 1999). Regression analyses were performed on numbers of whitefly landings on collards and Giant red mustard to test the hypothesis that landings will decrease with time on the collards and increase on the mustard. Numbers of whitefly landings were divided by leaf area for analysis and presentation. Landings per unit leaf area were analyzed as two separate 1-way ANOVAs (numbers of landings as dependent variable; host plants and paired combinations of host plants were treatments). Means were separated using Fisher's LSD test. Statistical analyses were performed using Systat 12 (Systat Software Inc., San Jose, CA).

RESULTS

Olfactometer experiment In the first bioassay, seven whiteflies selected the clean air control, 17 selected the collards, 1 was repelled and 5 showed "no decision" ($P = 0.108$). In the second bioassay, 8 adult female whiteflies selected the clean air control, 2 selected the mustard, 6 were repelled and 14 were designated as "no decision" ($P = 0.0042$). In the final bioassay, one female each entered the mustard and collard tubes, 17 were repelled and 11 were "no decision" ($P < 0.001$).

Outdoor potted experiment Only the collards and Giant red mustard were analyzed using regression because they contained sufficient data for analysis. Regression analysis on number of whitefly landings in the collards alone (control) did not show a significant decrease with time ($F = 2.7$; df = 1, 607; $P = 0.1$; $R^2 = 0.004$). Similarly, the regression on landings as a function of time in Giant red mustard was also insignificant ($F = 0.85$; df = 1, 124; $P = 0.36$; $R^2 = 0.006$). Leaf areas (mean ± SE) were significantly different (F=3.6; df = 3, 36; P< 0.05; $R^2 = 0.23$) among the test plants with the Tender green mustard (543.0 ± 65.4 sq cm) having the greatest leaf area, followed by the Giant red mustard (508.5 ± 60.1 sq cm) and collards (383.1 ± 50.7 sq cm). The Ragged leaf mustard had the smallest leaf area with a mean of 315.9 (± 46.0 sq cm). One-way ANOVA on host plant species showed a significantly higher number of landings on collards and lowest on the Giant red mustard ($F = 4.0$; df = 3, 134; $P < 0.01$; $R^2 = 0.08$) (Fig. 1). The 1-way ANOVA on the effects of plant combinations showed highest total numbers of whitefly landings on the collard + collard combination, and lowest on that of collard + Giant red mustard ($F = 3.5$; df = 3, 134; $P = 0.016$; $R^2 = 0.07$).

Numbers of eggs laid (per sq cm leaf area) was not significantly different among the different crop pair combinations ($F = 1.3$; df = 3, 134; $P = 0.28$; R^2 =0.03). However, numbers of eggs laid were higher on

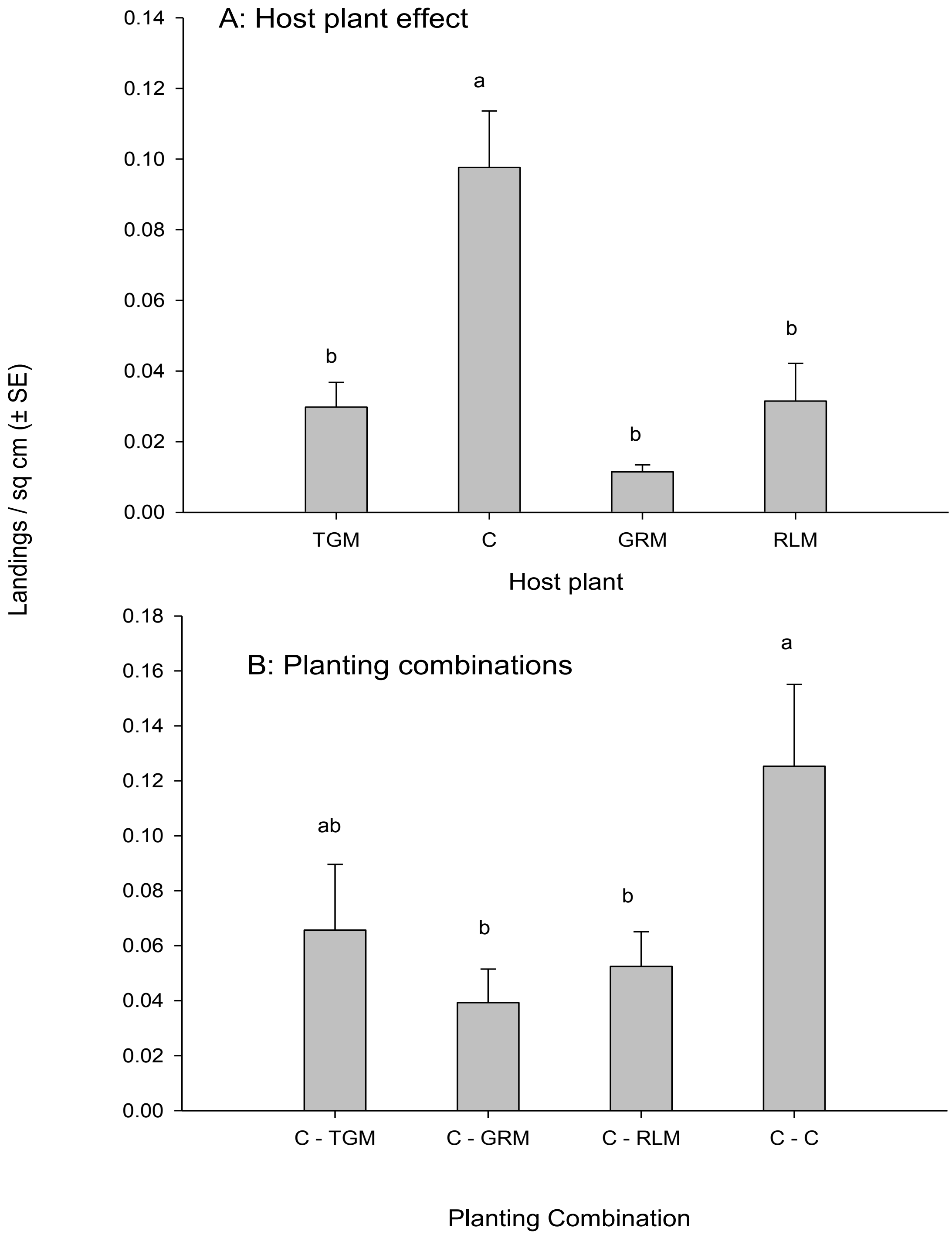

Fig. 1. Outdoor experiment – landing preference by *Bemisia argentifolii*. A) Numbers of landings (per sq cm ± SE) on host plant: TGM = Tender green mustard; C = collards; GRM = Giant red mustard; RLM = Ragged leaf mustard (F = 4.0; df = 3, 134; P < 0.01; R^2 = 0.08). B) Numbers of landings (per sq cm ± SE) by planting combinations. C-TGM = collard and Tender green mustard; C-GRM = collard + Giant red mustard; C – RLM = collard + Ragged leaf mustard; C-C = collard + collard combination (F = 3.5; df = 3, 134; P = 0.016; R^2 = 0.07). Identical letters indicate means that are not significantly different (P = 0.05; Fisher LSD).

the collard plant, as compared to any of the other mustard plant varieties (F = 3.8; df = 3, 134; P = 0.012; R^2 =0.08; Fisher LSD at P = 0.05) (Fig. 2).

DISCUSSION

Host location in whiteflies is largely dependent on visual cues (Mound 1962), but olfactory cues are also important (Bleeker et al. 2009). Although considered relatively weak fliers, they are capable of direct, active flight (Byrne 1999), with individuals typically making short interplant movements until encountering a suitable host (van Lenteren and Noldus 1990). Furthermore, whiteflies are known to possess olfactory receptors and are attracted to host plant odors (Visser 1986). These characteristics indicate that habitat manipulations could be used to reduce whitefly abundance in crops (Potting et al. 2005).

Previous research on trap crops as cultural controls has produced variable results. The presence of seemingly highly preferred hosts does not reduce whitefly abundance in less preferred target crops (Smith and McSorley 2000, Lee et al. 2009). Further, intercropping poor or non-host plants may not reduce populations in suitable crop hosts (Smith et al. 2001). In contrast, interplanting tomato with cucurbits can reduce the abundance of whiteflies and/or incidence of *Tomato yellow leaf curl virus* in tomato (Al-Musa 1982, Schuster 2004). Cotton with a cantaloupe trap crop had lower whitefly populations than unprotected cotton (Castle 2006). In turn, cover crops of buckwheat, *Fagopyrum esculentum* Moench (Caryophyllales: Polygonaceae), can reduce whitefly densities and incidence of squash silverleaf in zucchini (Hooks et al. 1998, Frank and Liburd 2005), but the effect may depend on overall pest abundance (Castle 2006, Manandhar et al. 2009). Weeds can also act as intercrops to reduce whiteflies in a crop (Showler and Greenberg 2003, Bezerra et al. 2004).

Different mechanisms could account for how plant diversity affects whitefly abundance. One direct

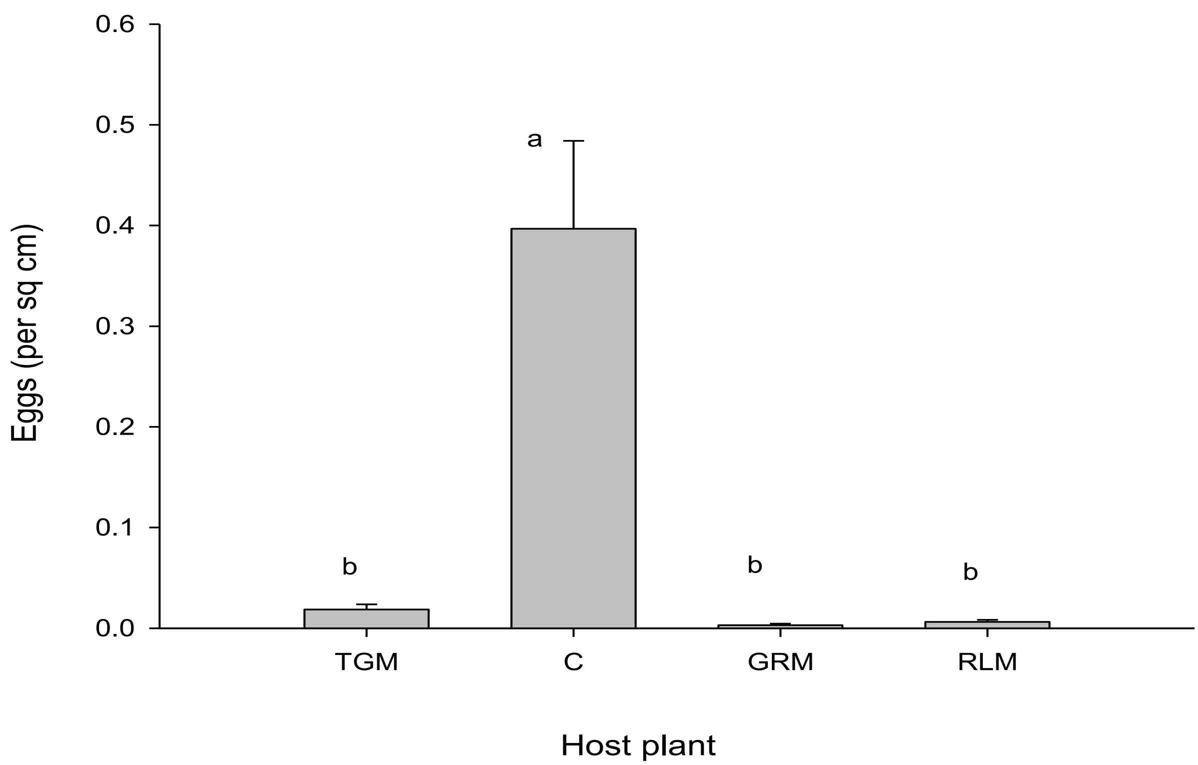

Fig. 2. Outdoor experiment – ovipositional preference by *Bemisia argentifolii*. Numbers of eggs per sq cm leaf area shown by host plant. TGM = Tender green mustard; C = collards; GRM = Giant red mustard; RLM = Ragged leaf mustard (F = 3.8; df = 3, 134; P = 0.012; R^2 = 0.08). Identical letters indicate means that are not significantly different (P = 0.05; Fisher LSD).

effect could stem from habitat diversity leading to sensory overstimulation of whiteflies, which disrupts behavior (Power 1991, Bernays 1999, Bird and Krüger 2006). Alternatively, specific plant characteristics, such as volatiles, could influence host selection. Certain terpenes extracted from wild tomato can repel whiteflies from otherwise acceptable cultivated tomato (Bleeker et al. 2009). Extracts and oils of certain other plants are also deterrent or repellent to adults when applied to tomato (Zhang et al. 2004, Al-mazra'awi and Ateyyat 2009).

Dual-port olfactometers, such as the Y-tube, are typically used in two modes of experiments: non-competitive and competitive (Bernier et al. 2007). In non-competitive mode, a treatment is compared simultaneously against a blank control, often clean air. In competitive mode, two treatments are compared simultaneously to gauge relative attraction of one over the other. Here, the tests using empty pots with moistened soil against either lacerated leaves of collards or mustard constituted non-competitive controls, whereas the test of lacerated collard against mustard leaves was a competitive test. The 2-tailed binomial test is a common method of statistical analysis in Y-tube olfactometer experiments (e.g., Belda and Riudavets 2010; Ibeas et al. 2008; Maeda and Liu 2006; Nakamuta et al. 2005).

In these experiments, we observed movement away from airflows, which we interpreted as "repellency". As in the case of movement towards an airflow, we used 7 cm as the minimum distance traversed to indicate either attraction or repellency. In the non-competitive tests, a reasonable expectation would be that the whiteflies would be attracted to the collards versus the clean air. However, when presented a choice between clean air and mustard leaves (competitive tests), whiteflies should be attracted to the clean air to avoid the mustard repellent volatiles. Although a higher number moved towards the collards in the 1st bioassay, the effect was not significant (P = 0.108). In the test between clear air and mustard leaves, only 2 selected the mustard and 14 selected either the clean air or were repelled (P =0.0042), suggesting the whiteflies were avoiding the mustard odors. In the final bioassay, 1 female selected the mustard tube and 18 either the collard or were repelled, possibly indicating strong aversion to the mustard odors (P < 0.001). We will conduct further studies on this apparent avoidance behavior of the whitefly using an I-tube or a single tube olfactometer to better determine the repellency of the mustard plant or a commercial mustard oil against whiteflies.

Although olfactometers are a promising tool in the study of whitefly host plant selection, the results found in this study are inconclusive. Volatiles may elicit only weak responses from the silverleaf whitefly, as evidenced by these results and the need to lacerate the leaves before whiteflies responded to the treatments. We found a possible weak positive response towards collards, which is not surprising given the oviposition preference reported in the outdoor experiment. However, demonstrating repellent effects using dual-port olfactometers is more difficult because of the number of insects designated as "no decision", and excluded from statistical analysis. In such cases, it is difficult to ascertain whether such insects are truly "repelled" or simply non-responsive (Bernier et al. 2007). Dual-port olfactometers and binomial tests may not be the most appropriate techniques with which to study repellency. Dogan and Rossignol (1999) actually modified an olfactometer based on measuring repellent effects through insect movement away from a treatment.

The outdoor experiment partially supported previous findings of preferential oviposition in collards and avoidance of Giant red mustard (Legaspi 2010). Collard plants received the highest numbers of whitefly landings per unit of leaf area, while giant red mustard had the lowest. Furthermore, the collard plants grouped together had the highest numbers of landings, and the collard + Giant red mustard combination, the lowest. However, these effects did not translate into differences in oviposition. Numbers of eggs laid per unit of leaf area were again highest on the collards alone, but there were no significant differences in numbers laid within the different collard - mustard combinations. Our results suggest that having a mustard companion crop did not appear to confer deterrence from whitefly oviposition to the collard crop.

Preferential oviposition by *B. argentifolii* has been demonstrated previously in field studies (e.g., Yee and Toscano 1996, Chu et al. 1995). Field counts of whitefly adults and nymphs were highest in squash (*Cucurbita pepo* L.) and cantaloupe (*Cucumis melo* L. *reticulatus*), intermediate on broccoli (*Brassica oleracea* L. var. *italica*), collards and cabbage (*Brassica oleracea* L. var. *capitata*), and lowest in giant red mustard (Legaspi 2010). High per-plant densities of whitefly adults or immature lifestages on squash indicate that it may be a useful trap crop, preventing whitefly adults from moving to a target crop such as collards (Legaspi 2010) or tomato (Schuster 2003).

In summary, our olfactometer experiments did not prove attraction of whiteflies to collards, but indicated possible aversion to mustard volatiles. However, modifications to dual port olfactometers may be needed to produce more conclusive results. With regards to use of the mustard plant as a companion crop to control whiteflies in collards, the results of our outdoor experiment did not show clear evidence of deterrence against whiteflies. Whitefly landings may be reduced when collards are planted with mustards, but we found no reduction in oviposition. We are currently testing commercial mustard oils in no-choice tests in cantaloupe potted plants in the laboratory. Preliminary results indicate significantly lower number of whitefly eggs laid in plants sprayed with 3% mustard oil versus the water control (Legaspi, unpublished data). Thus, the potential for use of mustard plants may be as an oil formulation to spray target crops against whiteflies. Further testing of the plant compounds that may be responsible for deterrence against whiteflies is warranted.

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