

A Preliminary Investigation of Giant Red Mustard (*Brassica juncea*) as a Deterrent of Silverleaf Whitefly Oviposition¹

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Abstract Different pairs of plants planted in a single pot were tested in the greenhouse for oviposition preference by the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Hemiptera: Aleyrodidae). Treatments were: 2 giant red mustard plants (*Brassica juncea* (L.) Czern.), 2 collards (*Brassica oleracea* L. var. *acephala*), and 1 plant of each species in individual pots. Treatments were exposed to whitefly adults and numbers of eggs laid were counted 6 d later. Numbers of whitefly eggs were significantly lower on the mustard-mustard treatment. Average egg counts were lower on collard plants in the treatment where both host plants were presented simultaneously than in treatments where collards were presented alone. These results suggest the possibility of repellent volatiles in the giant red mustard. To test for repellent effects in the field, an experimental plot was planted with squash (*Cucurbita pepo* L.), cabbage (*Brassica oleracea* L. var. *capitata*), broccoli (*Brassica oleracea* L. var. *italica*), collards, and cantaloupe (*Cucumis melo* L. *reticulatus*). A central plot of mustard transected the experimental area. To measure any effects of distance from the mustard, weekly sampling was performed at 5 equidistant intervals of 2.4 m to a distance of 12.2 m from the central mustard plot. Results showed whitefly attraction to squash and cantaloupes and aversion to mustard, with other crops (including collards), hosting intermediate insect densities. Repellent properties of mustard at these sampling distances in the field did not affect attraction or oviposition on other crops. It is possible that the sampling distances were too large to detect any repellent effects, or any effects of volatiles were stronger within the confines of the laboratory test arena. In general, results presented in this study are preliminary. Further field research needs to be conducted to determine if intercropping giant red mustard can be a promising strategy. However, squash and cantaloupe may have potential as trap crops for whitefly, or giant red mustard may be planted as a resistant crop when heavy whitefly infestations are anticipated.

Key Words intercropping, repellent crops, trap crops, vegetable crops, *Bemisia argentifolii*

The use of companion crops with repellent or masking volatiles have long been suggested as a potential crop protection method that disrupts insect pest host-finding behavior (Feeny 1976, Tahvanainen and Root 1972, Root 1973, Uvah and Coaker 1984). More recently, the use of repellent crops has been incorporated into the concept of “association resistance” (Held et al. 2003), or as the “push” component in “push-pull” pest control strategies (Pickett et al. 2006, Khan et al. 2008). In the laboratory, olfactometer studies have shown that insect pests may indeed display less attraction to host plant odors when mixed with those of a nonhost plant. The odor of potato

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plants (*Solanum tuberosum* L.) induced positive anemotaxis in Colorado potato beetles, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). However, the response was neutralized when potato plant odors were mixed with those of nonhost wild tomatoes (*Lycopersicon hirsutum* f. *glabratum* C.H. Mull) or cabbage (*Brassica oleracea* L. var. *gemmifera* DC) (Thiery and Visser 1986, 1987). Fly movements were studied in the laboratory on cabbage root flies (*Delia radicum* L.) (Diptera: Anthomyiidae) landing on host cabbage (*B. oleracea* var. *capitata*), nonhost clover (*Trifolium subterraneum* L.), and on cabbage surrounded by clover (Morley et al. 2005). Protracted time spent on the nonhost plants was determined to be the factor that interfered with host plant finding in diverse plantings, rather than repellent effects as suggested in earlier studies.

Field experiments using companion crops with nonhost masking odors have yielded mixed results. Three reputedly effective companion species—rue (*Ruta graveolens* L.), zonal geranium (*Pelargonium X hortorum* Bailey), and garlic chives (*Allium schoenoparum* L.)—were interplanted with roses (*Rosa X hybrida* "Ultimate Pink") for protection against the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) (Held et al. 2003). However, comparison against rose-only control plots showed no significant decreases in numbers of Japanese beetles on roses. Interplanting with geranium actually increased beetle numbers on the roses, suggesting that companion planting with reputedly repellent plants was an ineffective control strategy (Held et al. 2003). Field trials using 4 varieties of barley showed reductions in aphids (*Rhopalosiphum padi* (L.) [Hemiptera: Aphididae]) when used in proper varietal combinations (Ninkovic et al. 2002). In Egypt, cotton (*Gossypium barbadense* L.) was intercropped with the putative repellent, basil (*Ocimum basilicum* L.), in a field trial. The cotton-basil intercropping significantly reduced total pest infestation and led to a 50% reduction in abundance of the pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) (Schader et al. 2005). In field trials, bean flower thrips (*Megalurothrips sjostedti* Trybom [Thysanoptera: Thripidae]) oriented and settled on pure cowpea (*Vigna unguiculata* (L.) Walp) at higher densities than on cowpeas intercropped with nonhost maize (Kyamanywa et al. 1993). Volatiles from nonhost plants may interfere with the attractiveness of cowpea leaf buds, resulting in "olfactory masking" (Koschier 2006).

Similar field trials in Nigeria showed significantly lower populations of aphids (*Aphis craccivora* Koch, [Hemiptera: Aphididae]) and thrips (*M. sjostedti*) in cowpea + sorghum intercrops compared with pure stands of cowpea. Inconclusive results in 2 seasons were obtained for the maruca pod borer, *Maruca vitreta* F. (Lepidoptera: Crambidae) (Hassan 2009). Aphids and thrips populations were significantly reduced in the cowpea + sorghum intercrops in field trials in Uganda but were higher in the cowpea + greengram, *Vigna radiata* (L.) R. Wilczek (Nampala et al. 2002). In contrast, maruca pod borer and pod sucking bug infestations and their associated damage were significantly higher in the cowpea + sorghum intercrop than in the other cropping systems, thus indicating that not all intercropping systems result in reduced pest infestations.

Conflicting results have been reported on the use 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 emission of volatile compounds from tomato. Similar studies on tomato + cabbage and tomato + fava bean (*Vicia faba* L.) intercrops showed no effects of population levels on the moth (Chelliah and Srinivasan 1986, Badenes-Perez et al. 2005).

Here, we compare oviposition preference in the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Hemiptera: Aleyrodidae) = *Bemisia tabaci* (Gennadius) in the

mustard as a repellent intercrop by recording whitefly counts at different distances from a central row of mustard.

Materials and Methods

Greenhouse experiment. Pairs of host plants were grown in single 3.78-L plastic pots and maintained in a greenhouse free from whitefly infestation. Plant pair treatments in individual pots were: (1) collard (*Brassica oleracea* L. var. *acephala*) + collard, (2) mustard + collard, and (3) mustard + mustard. When plants had grown to a height of ~45 cm, they were randomly placed on a 1-m tall metal greenhouse table, 76 cm apart from the nearest plant. At the beginning of the experiment a collard plant infested with *Bemisia* was placed at a distance of 1 m from each end of the group of pots. In addition ~1250 *Bemisia* adults were released from vials at the base of each of these infested plants. There were 8 replicate pots for each of the 3 plant-pairing treatments resulting in a total of 24 pots per trial. The trial was conducted twice (3 plant pair combinations \times 8 replicates \times 2 trials = 48 pots total). After 6 d a single leaf was randomly sampled from each plant, and eggs and nymphs were counted in the laboratory using a stereo microscope.

Field experiment. Experiments were conducted in the fall of 2008 at the USDA-ARS, CMAVE, Center for Biological Control in Tallahassee, FL (30°26'17" N, 84°16'50" W). An experimental plot was planted where squash (*Cucurbita pepo* L.), cabbage (*Brassica oleracea* L. var. *capitata*), broccoli (*Brassica oleracea* L. var. *italica*), collards, and cantaloupe (*Cucumis melo* L. *reticulatus*) were grown in 12 m double rows abutting adjacent 6 m sections of red mustard from either side. Four replicate rows of each given vegetable variety were planted in beds that were 1.2 m wide and supplied with plastic irrigation tape to provide water and liquid fertilizer. On 2 September, cabbage, broccoli, and collards were planted in the field from transplants previously started in a greenhouse. Cantaloupe, squash and giant red mustard were planted from seed. Standard agronomic practices in the region were followed.

Each week, leaves were sampled to determine infestation by *B. argentifolii*. Samples were taken at 5 equidistant intervals of 2.4 m to a distance of 12.2 m within each vegetable crop row and every 1.5 m in the mustard rows. Before leaves were removed from the plant, they were visually inspected and counts were made of adult whiteflies present on the leaves. Leaves were brought back to a laboratory, and counts were made of immature *B. argentifolii* using a stereo microscope. Counts of *B. argentifolii* nymphs were made by randomly subsampling a given leaf in 4 separate 2.5 \times 2.5 cm squares.

Statistical analysis. The greenhouse experiment was analyzed as a 2-way ANOVA where number of eggs was the dependent variable, and treatments were plant host and combination used (i.e., collard-collard, mustard-collard, mustard-mustard). Plant host and combination treatments were analyzed separately using 1-way ANOVA. In the case of plant combination treatments, means were separated using Tukey's HSD ($P < 0.05$). In the field experiment, the effect of host plant on whitefly adult and immatures was tested using a 1-way ANOVA with host crop as the treatment effect. Means were separated using Tukey's HSD ($P < 0.05$). The effect of sampling distance was tested using a regression analysis where insect counts (adult or immature) were the dependent variables and distance from the mustard crop was the independent variable. All statistical analyses were performed using Systat 12 (Systat Software Inc., San Jose, CA).

Results

The greenhouse oviposition preference experiment showed significant effects of both plant species and the plant pair treatments when analyzed together (2-way ANOVA: $F = 10.5$; $df = 3, 140$; $R^2 = 0.18$; $P < 0.001$) (Fig. 1). When plant pairing effect was analyzed separately, significant treatment effects also were found ($F = 14.7$; $df = 2, 141$; $R^2 = 0.17$; $P < 0.001$). The collard + collard treatment had significantly more whitefly eggs than the mustard + collard and mustard + mustard treatment combinations (Tukey HSD, $P < 0.05$). When host plant was analyzed separately, significantly more whitefly eggs were laid on collard than on mustard plants (1-way ANOVA: $F = 26.4$; $df = 1, 142$; $R^2 = 0.16$; $P < 0.001$).

In the field experiment, adult and immature whitefly counts during the season were highest in the squash and cantaloupe (Fig. 2) and lowest in the mustard (Fig. 3). Insect sampling in the squash and cantaloupe were terminated on 27 October and 3 November, respectively because of crop loss due to whitefly damage as well as resulting cucurbit leaf crumple virus infections (S. Adkins, pers. comm.). When analyzed over the entire season, whitefly adult counts were highest in squash and cantaloupe, followed by broccoli and collards (Fig. 4). Lowest adult counts were in cabbage, then mustard ($F = 91.1$; $df = 5, 4338$; $R^2 = 0.095$; $P < 0.01$; Tukey HSD, $P < 0.05$; Fig. 4A). Whitefly immatures were affected similarly by host crops ($F = 174.8$; $df = 5, 4254$; $R^2 = 0.17$, $P < 0.01$; Tukey HSD, $P < 0.05$; Fig. 4B). Regression analyses of whitefly adults and immatures showed

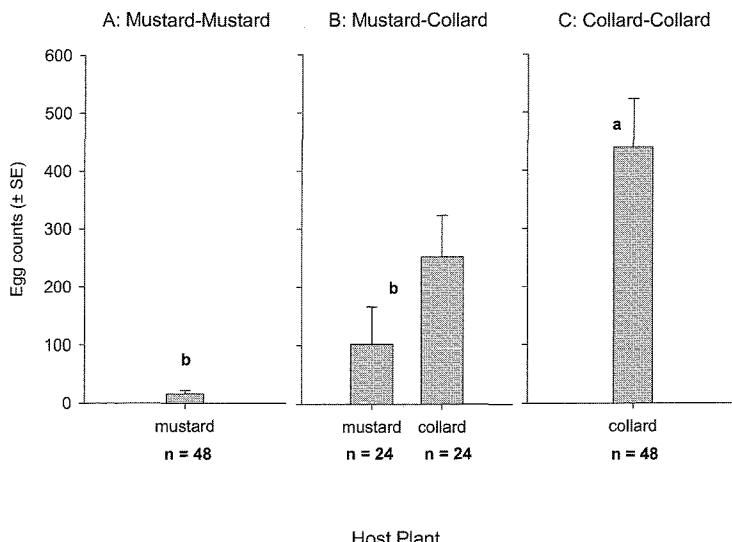


Fig. 1. Greenhouse experiment on oviposition preference by *Bemisia argentifolii* for collards and giant red mustard. A) experimental arena where both plants were mustard; B) plant pairing had 1 mustard and 1 collard plant; C) both plants were collards (n indicates number of plants used to calculate mean shown). Letters indicate treatment differences (Tukey HSD, $P < 0.05$)

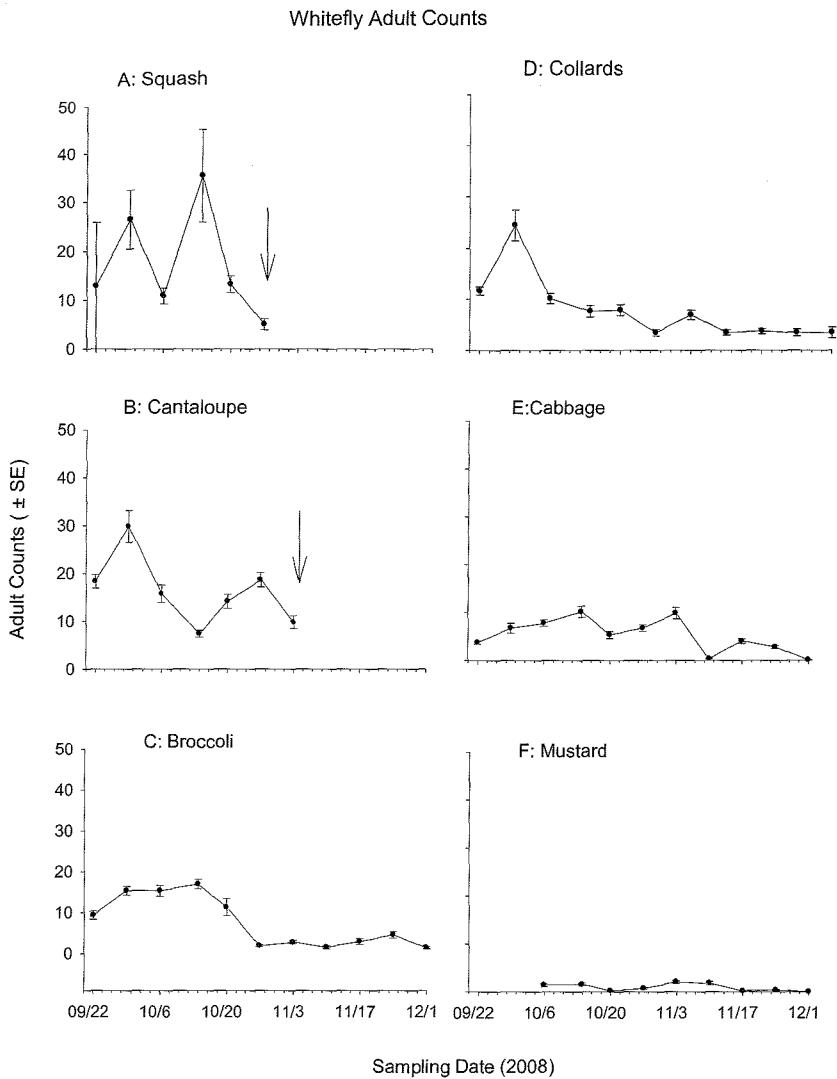


Fig. 2. Adult whitefly counts (mean \pm SE) with time for each crop. Arrows in A) and B) indicate points at which insect sampling was halted because of crop death due to whitefly pressure and cucurbit leaf crumple virus disease.

no significant effects of sampling distance from the mustard crop (adults: $F = 0.14$; $df = 1, 4342$; $R^2 < 0.01$; $P = 0.71$; immatures: $F = 2.89$; $df = 1, 4258$; $R^2 < 0.01$; $P = 0.09$).

Discussion

The greenhouse experiment showed that *B. argentifolii* preferred to lay eggs in collards and that the effect was

Whitefly Immature Counts

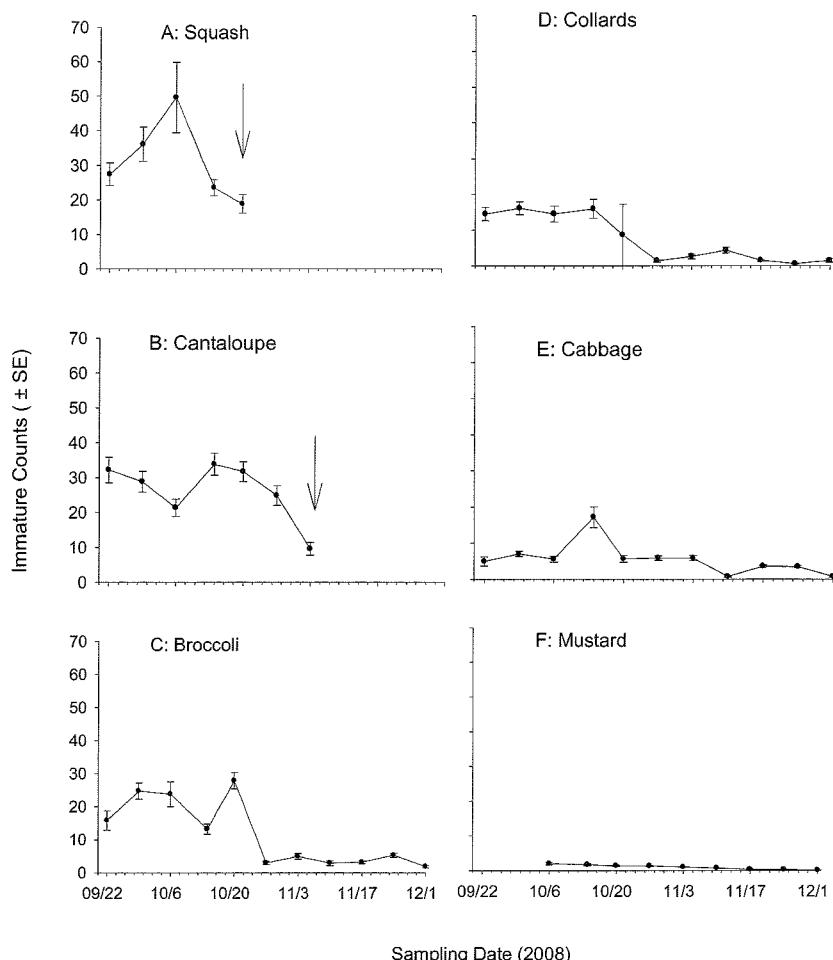


Fig. 3. Immature whitefly counts (mean \pm SE) with time for each crop. Total immature counts indicate sums of eggs, larvae and pupae at each sampling date. Arrows in A) and B) indicate points at which insect sampling was halted because of crop death due to whitefly pressure and cucurbit leaf crumple virus disease.

together. Even when presented with collard and mustard plants together, oviposition was higher on the collards. These results clearly indicate preferential oviposition on collards, and a possibility of repellence by mustard. The use of whiteflies reared on collards may have induced some preference for collards in this experiment because

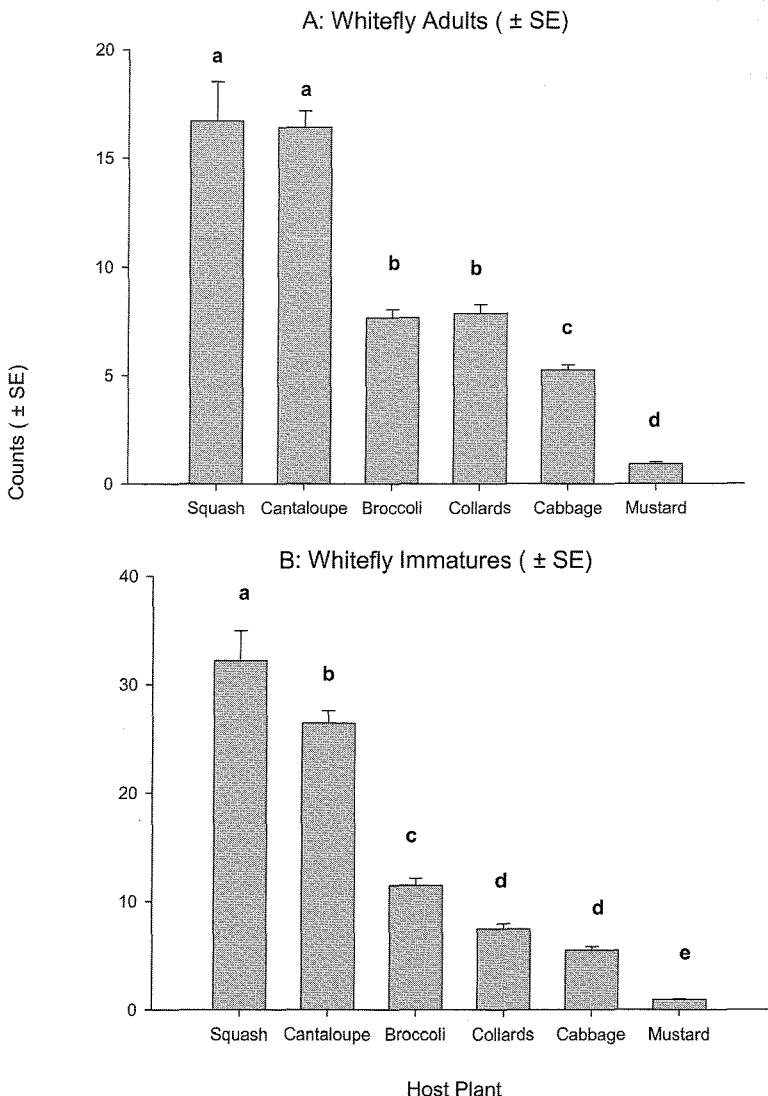


Fig. 4. Seasonal means for A) adult whiteflies and B) immature whiteflies for each crop. Different letters indicate means were significantly different (Tukey HSD, $P < 0.05$).

and experience. Wild Queensland fruit flies (*Bactrocera tryoni* (Froggatt) (= *Dacus tryoni* [Diptera: Tephritidae])) displayed preferential oviposition to specific host fruits (e.g., pear) after 3 d exposure as compared with other host fruit types (e.g., tomato, grape) (Prokopy and Fletcher 1987). Moreover, the effects were shown to be reversible. Experience may, in fact, cause nonhost plants to become attractive to phytopha-

Wang et al. (2008) found that whereas naïve females showed aversion to nonhost plant odors, experienced females were no longer repelled or even became attracted to the odor. However, it is unlikely that previous conditioning had a significant role in the aversion of *Bemisia* to mustard because the same effect was found in the field experiments.

Preferential oviposition by *B. argentifolii* has been demonstrated in the field by previous authors. Lower densities of whitefly eggs and nymphs were recorded on alfalfa than on broccoli, zucchini, cantaloupe, and cotton (*Gossypium hirsutum* L.) in a field experiment in the Imperial Valley, CA (Yee and Toscano 1996). In field and greenhouse tests, the whitefly displayed a preference for cantaloupe to cotton, broccoli, and lettuce (*Lactuca sativa* var. *capitata* L.) (Chu et al. 1995). High per-plant densities of whitefly adults or immature life stages on squash relative to tomatoes indicate that squash may be a useful trap crop, preventing whitefly adults from moving to tomato (Schuster 2003).

Field experiments described here showed whitefly adult attraction to squash and cantaloupes and aversion to giant red mustard. Other crops planted were intermediate. The preferential attraction resulted in preferential oviposition in squash and cantaloupe, as well as aversion to giant red mustard. However, we found no significant measurable effects of plant volatiles with distance. If giant red mustard plant volatiles had a repellent effect on whiteflies, adult and egg counts would be expected to increase with distance from the central giant red mustard plot. It is possible that repellent effects may be shown at a closer proximity to the giant red mustard plants than the 0.4-m sampling distance.

Indian mustard (*Brassica juncea* (L.) Czern.) was evaluated as a trap rather than repellent intercrop for cabbage (*Brassica oleracea* var. *capitata* L.) in West Texas (Bender et al. 1999). Insects were monitored in pure cabbage, cabbage surrounded by Indian mustard, and the Indian mustard intercrop. Intercropping did not affect number of lepidopterous larvae in cabbage and was not a promising management practice under normal pest infestation levels. Mustard may also be the target rather than the companion crop. In India, aphids (*Lipaphis erysimi* Kaltenbach) are a pest of mustard (*B. juncea* "Rohini"). Aphid infestations were compared in a monocrop against intercrops with artemisia (*Artemisia annua* L.), coriander (*Coriandrum sativum* L.), chamomile (*Matricaria chamomilla* L.), fennel (*Foeniculum vulgare* Mill.), and dill (*Anethum sowa* Kurz.). The fennel intercrop showed significant declines in aphid infestation levels (Singh and Kothari 1997). Field experiments in Bangladesh showed that intercropping mustard (*Brassica napus* "Bari Sarisha-7") with onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) significantly reduced infestations of the aphid (*L. erysimi*) (Sarker et al. 2007). Over 2 growing seasons, economic return was most favorable in the mustard+garlic intercrop and lowest in the mustard monoculture. In central Guatemala, common bean (*Phaseolus vulgaris* L.) was intercropped with poor and nonhost crops to evaluate control against whitefly pests (*Trialeurodes vaporariorum* Westwood and *B. tabaci*). Data interpretation was complicated by high variability and reduced crop quality. No evidence was found that intercropping affected whitefly populations, regardless of infestation level or season (Smith et al. 2001).

In summary, our greenhouse experiments showed reduced oviposition by *B. argentifolii* on giant red mustard. Furthermore, pairs of collard plants showed higher egg numbers than pairs of giant red mustard plants, or a pair of mustard + collard plants, suggesting plant volatiles may have attracted whitefly adults to collards or repelled them from giant red mustard. The field experiments showed whitefly attraction to

squash and cantaloupes and aversion to giant red mustard, with other crops (including collards) with intermediate insect densities. Whereas giant red mustard was shown to reduce oviposition on associated collard plants in the greenhouse, this effect was not seen in the field. It is possible that the sampling distances were too large to detect any repellent effects, or any effects of volatiles were stronger within the confines of the greenhouse test arena. In general, results presented in this study are preliminary. Further field research needs to be conducted to determine if intercropping giant red mustard can be a promising strategy. However, squash and cantaloupe may have potential as trap crops for whitefly, or giant red mustard may be planted as a resistant crop when heavy whitefly infestations are anticipated.

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