Research Article

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Preference of *Bemisia tabaci* biotype B on zucchini squash and buckwheat and the effect of *Delphastus catalinae* on whitefly populations

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Abstract

BACKGROUND: Zucchini squash, *Cucurbita pepo* L., is an important vegetable crop in Florida. Physiological disorders and insect-transmitted diseases are major problems for squash growers in semi-tropical regions around the world. *Bemisia tabaci* (Gennadius) biotype B is a significant whitefly pest and is largely responsible for transmitting viruses and causing physiological disorders in squash. Several studies have shown that whitefly populations are reduced when crops are interplanted with non-host cover crops or mulches. The aim of the present study was to determine how the presence of buckwheat, *Fagopyrum esculentum* Moench, and a key predator, *Delphastus catalinae* (Horn), affect whitefly colonization on squash.

RESULTS: Whitefly densities were higher on squash than on buckwheat. The introduction of *D. catalinae* on squash significantly reduced whitefly populations. Overall, there were higher densities of *D. catalinae* on squash where the whitefly pest was more concentrated compared with buckwheat.

CONCLUSION: The study provided preliminary evidence that *D. catalinae*, when used in conjunction with buckwheat as a living mulch, may aid in reducing whiteflies in squash. This greenhouse experiment highlights the need to investigate a multitactic approach of intercropping buckwheat with squash and the incorporation of *D. catalinae* in the field to manage populations of whiteflies and whitefly-transmitted diseases.

Keywords: *Bemisia tabaci* biotype B; zucchini squash; living mulch; buckwheat; *Delphastus catalinae*

1 INTRODUCTION

Zucchini squash, *Cucurbita pepo* L., is a high-value vegetable crop in Florida.[1] However, plant physiological disorders and plant viruses transmitted by *Bemisia tabaci* (Gennadius) biotype B are serious problems for many squash growers in Florida and other semi-tropical regions around the world. One of the most damaging plant physiological disorders in squash is squash silverleaf (SSL) disorder, which is associated with the feeding of immature whiteflies.[2,3] SSL is characterized by silvering of the upper leaf surface and blanching of fruit, which can reduce the quality of the fruit produced, depending on the severity of the disorder.[4−6] In addition to plant physiological disorders, *Cucurbit leaf crumple virus* is an important whitefly-transmitted virus that was first recorded in Florida during the fall of 2006 and has the potential to cause significant yield losses in squash.[7,8]

Several studies have shown that whitefly populations are reduced in mixed cropping systems and in crops interplanted with non-host cover crops or mulches.[9−11] Living mulches reduce whitefly densities on host plants by reducing the contrast between bareground and the plant canopy.[12] Additionally, non-host crops planted within the same field as the cash crop can serve as habitats for conserving and increasing populations of natural enemies, thereby introducing diversity into agroecosystems for improved pest control.[13]

The coccinellid beetle *Delphastus catalinae* (Horn) has been cited as a good biological control candidate for whiteflies.[14,15] *D. catalinae* is an obligate whitefly predator with high prey consumption rates. Larvae are known to consume an average of 167 eggs per day and up to 1000 eggs before pupating.[7] *D. catalinae* also exhibits long adult lives and high fecundity rates.[14] Legaspi *et al.*[17] observed that *D. catalinae* displayed a preference for whiteflies in the egg stage, followed by small then large nymphs. They suggested that *D. catalinae* would be effective early in the season when eggs are abundant. Liu and Stansly[15] observed that some *D. catalinae* larvae fed on honeydew and dew drops, and the availability of alternate food sources may enhance survival and discourage dispersal of the natural enemy.

Buckwheat, *Fagopyrum esculentum* Moench, has been cited as an important living mulch in cucurbit production systems.[9,10] Significant reductions in pest densities were recorded when buckwheat was intercropped into squash production systems when compared with zucchini in bare-ground treatments.[9,10] Buckwheat is an annual plant that completes its life cycle in Florida in 6 weeks.[9] Buckwheat also flowers profusely and attracts beneficial insects

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to the cucurbit crop.[9,13] Attraction of natural enemies of whiteflies may be an important advantage of implementing buckwheat mulches because natural enemies can play an important role in pest reduction.

The implementation of cultural control techniques in agriculture, such as the augmentation and conservation of biological control and the establishment of living mulches, has the potential to reduce whitefly numbers as well as the incidence of SSL disorder and whitefly-transmitted viruses in cucurbits. The purpose of this study was to investigate the effect of a living mulch and a natural enemy on *B. tabaci* biotype B population densities in zucchini squash. The specific objectives were: (1) to evaluate the effect of buckwheat as a living mulch on whitefly density when interplanted with zucchini squash; (2) to assess the impact of *D. catalinae* on whitefly population density in buckwheat and zucchini squash.

2 EXPERIMENTAL METHODS

Research was conducted during the spring of 2011 and 2012 in the Small Fruit and Vegetable IPM (SFVIPM) greenhouse at the University of Florida in Gainesville, Florida.

2.1 Insect colonies

Adult whiteflies and *D. catalinae* used in the trials were obtained from a colony reared in 30×30 cm wire mesh cages on collards, *Brassica oleraceae*, in the SFVIPM laboratory. *D. catalinae* adults were originally purchased from Bicontrol Network, LLC (Brentwood, TN) and maintained on a colony of *B. tabaci* biotype B for several months. The colony was maintained at 28 °C with $70 \pm 5\%$ RH on L:D 14:10 in the Department of Entomology and Nematology, University of Florida, Gainesville, Florida. Plants were watered 2–3 times per week to maintain turgidity, and new plants were put into the cage once every 2 weeks.

Squash variety 'Cashflow' (Johnny's Selected Seeds, Winslow, ME) and buckwheat plants were grown in the greenhouse in 1 L pots. Zucchini squash seeds were directly seeded by hand, whereas buckwheat seeds were first sown in a seed tray and hand transplanted after 2 weeks into 1 L pots containing organic garden soil (Miracle-Gro, Marysville, OH). Squash plants were fertilized with organic fertilizer (Scotts Organic Fertilizer, Marysville, OH).

The experiment was a split-plot design with four replicates to test the effects of a predator, *D. catalinae*, on whitefly populations on zucchini squash and buckwheat. The main plot treatments were presence or absence of *D. catalinae*, and subplot treatments were zucchini squash and buckwheat. Choice tests were conducted to determine preference of *B. tabaci* biotype B when exposed to zucchini squash and buckwheat. Two 1 m^3 locally made whitefly exclusion cages containing the zucchini squash and buckwheat plants were used for this study. Each cage contained eight plants, such that four zucchini squash plants and four buckwheat plants were distributed randomly within each cage. Cages 1 and 2 were infested with 25 adult whiteflies cage^{-1} on 8 March 2011 and 27 March 2012. The adult whiteflies were allowed to reproduce for approximately 1 week before cage 1 was infested with adult *D. catalinae*. Initially, 30 *D. catalinae* adults were released on 16 March 2011 to observe the effect of predation on whitefly population densities. During pretrial observations of *D. catalinae*, adult beetles demonstrated high dispersal rates from collards infested with whiteflies. Therefore, we infested cage 1 with a high number of *D. catalinae* adults relative to the number of whiteflies present in the cage. However, the following year the release of *D. catalinae* was reduced to 10 adults cage^{-1} to approximate a 1:10 predator to prey ratio. Cage 2 (control) did not receive any *D. catalinae* and only contained whiteflies.

2.2 Sampling

Sampling was conducted every 3 days for approximately 6 weeks from 11 March 2011 to 22 April 2011, and from 30 March 2012 to 7 May 2012. All the leaves on squash and buckwheat plants were examined using the leaf-turn method[1] to count adults. A 10× hand lens was used to quantify the number of whitefly first instars and second to fourth instars on each plant. Squash and buckwheat plants in cage 1 were visually inspected for the presence of *D. catalinae* adults, and their numbers were recorded. During the sampling period, there were approximately 6–8 leaves per squash plant.

2.3 Statistical analysis

Data were analyzed using the repeated-measures analysis of variance procedure (ANOVA; PROC GLM)[18] to investigate insect population density over time. Sample date was the repeated measure and treatment means were separated by the least significant differences (LSD) test.[18] Differences among treatments were considered to be significant if $P \leq 0.05$.

3 RESULTS

In 2011, adult whitefly densities were different between plant species ($F = 101.49$; df = 1, 194; $P \leq 0.0001$) and *D. catalinae* treatments ($F = 22.15$; df = 1, 194; $P \leq 0.0001$), and there was an interaction effect ($F = 19.79$; df = 1, 194; $P \leq 0.0001$). Adult whitefly densities were greater on zucchini squash compared with buckwheat (Fig. 1). Fewer adult whiteflies were recorded on squash in treatments with *D. catalinae* compared with treatments without *D. catalinae*. There was no significant difference in adult whitefly densities on buckwheat when comparing the *D. catalinae* treatments (Fig. 1). Adult whitefly densities were also different over time ($F = 14.12$; df = 14, 194; $P \leq 0.0001$), and there was a plant species × time interaction effect ($F = 14.10$; df = 14, 194; $P \leq 0.0001$), such that treatment differences were observed in the final 2 weeks of sampling. There was also a predator × time interaction effect ($F = 3.09$; df = 14, 194; $P = 0.0002$), such that treatment differences were observed in the final 2 weeks of sampling.

In 2012, there were adult whitefly density differences between plant species ($F = 90.77$; df = 1, 181; $P \leq 0.0001$) and *D. catalinae* treatments ($F = 56.37$; df = 1, 181; $P \leq 0.0001$), and there was an interaction effect ($F = 35.50$; df = 1, 181; $P \leq 0.0001$). Similarly to 2011, more adult whiteflies were recorded on zucchini squash than on buckwheat (Fig. 2). In addition, fewer adult whiteflies were recorded on plants treated with *D. catalinae* compared with treatments without *D. catalinae* (Fig. 2). Adult whitefly densities increased at varying rates for each treatment over time ($F = 11.37$; df = 13, 181; $P \leq 0.0001$), and there was a plant species × time interaction effect ($F = 9.38$; df = 13, 181; $P \leq 0.0001$), such that adult density differences among treatments were observed in the final 2 weeks of sampling. There was also a predator × time interaction effect ($F = 6.51$; df = 13, 181; $P \leq 0.0001$), such that treatment differences were observed in the final 2 weeks of sampling.

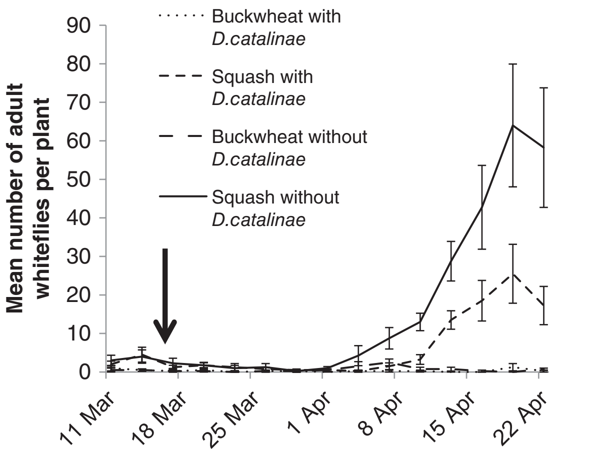

Figure 1. Mean (± SE) number of adult whiteflies observed for the whitefly preference study in spring 2011.[a]

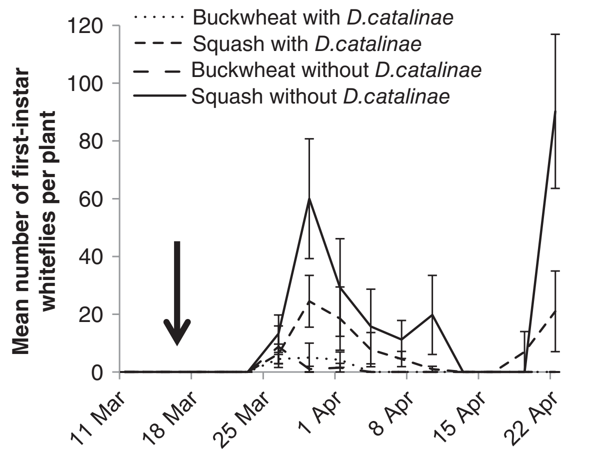

Figure 3. Mean (± SE) number of first-instar immature whiteflies observed for the whitefly preference study in spring 2011.[a]

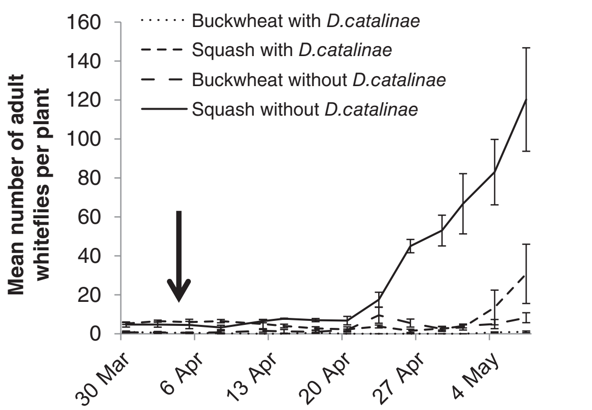

Figure 2. Mean (± SE) number of adult whiteflies observed for the whitefly preference study in spring 2012.[a]

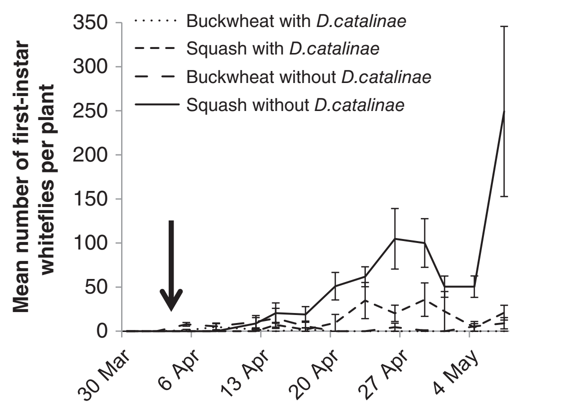

Figure 4. Mean (± SE) number of first-instar immature whiteflies observed for the whitefly preference study in spring 2012.[a]

In 2011, first-instar immature whitefly densities were different between plant species ($F = 34.39$; df = 1, 194; $P \leq 0.0001$) and *D. catalinae* treatments ($F = 7.94$; df = 1, 194; $P = 0.0053$), and there was an interaction effect ($F = 8.49$; df = 1, 194; $P = 0.0040$). There were more first-instar immature whiteflies on zucchini squash than on buckwheat (Fig. 3). Fewer first-instar immature whiteflies were recorded on zucchini squash in treatments with *D. catalinae* compared with treatments without *D. catalinae*; however, there was no significant difference in whitefly densities on buckwheat when comparing the *D. catalinae* treatments (Fig. 3). First-instar immature whitefly densities were also different over time ($F = 6.88$; df = 14, 194; $P \leq 0.0001$), and there was a plant species × time interaction effect ($F = 6.11$; df = 14, 194; $P \leq 0.0001$), such that treatment differences were observed in the third and sixth week of sampling. There was also a predator × time interaction effect ($F = 2.00$; df = 14, 194; $P = 0.0193$), such that treatment differences were observed in the third and sixth week of sampling.

In 2012, first-instar immature whitefly densities were different between plant species ($F = 38.54$; df = 1, 181; $P \leq 0.0001$) and *D. catalinae* treatments ($F = 22.69$; df = 1, 181; $P \leq 0.0001$), and there was an interaction effect ($F = 15.37$; df = 1, 181; $P = 0.0001$). There were more first-instar immature whiteflies on zucchini squash than on buckwheat (Fig. 4). Fewer first-instar immature whiteflies were recorded on zucchini squash in treatments with *D. catalinae* compared with treatments without *D. catalinae*; however, there was no significant difference in whitefly densities on buckwheat when comparing the *D. catalinae* treatments (Fig. 4). First-instar immature whitefly densities were also different over time ($F = 4.84$; df = 13, 181; $P \leq 0.0001$), and there was a plant species × time interaction effect ($F = 4.98$; df = 13, 181; $P \leq 0.0001$), such that treatment differences were observed from the third week until the sixth week of sampling. There was also a predator × time interaction effect ($F = 3.19$; df = 13, 181; $P = 0.0002$), such that treatment differences were observed from the fourth until the sixth week of sampling.

In 2011, second- to fourth-instar immature whitefly densities were different between plant species ($F = 33.39$; df = 1, 194; $P \leq 0.0001$) and *D. catalinae* treatments ($F = 26.18$; df = 1, 194; $P \leq 0.0001$), and there was an interaction effect ($F = 8.75$; df = 1, 194; $P = 0.0035$). There were fewer second- to fourth-instar immature whiteflies on zucchini squash in treatments with *D. catalinae* compared with treatments without *D. catalinae* (Fig. 5). There were also fewer second- to fourth-instar immature whiteflies on buckwheat in treatments with *D. catalinae* compared with treatments without *D. catalinae* (Fig. 5). Second- to fourth-instar immature whitefly densities were also different over time ($F = 4.10$; df = 14, 194; $P \leq 0.0001$), and there was a plant species × time interaction effect ($F = 2.59$; df = 14, 194; $P = 0.0019$), such that treatment

[a] Arrow indicates when *D. catalinae* was released

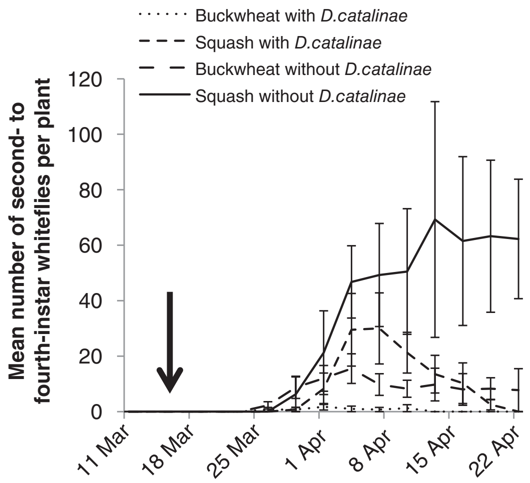

Figure 5. Mean (± SE) number of second- to fourth-instar immature whiteflies observed for the whitefly preference study in spring 2011.[a]

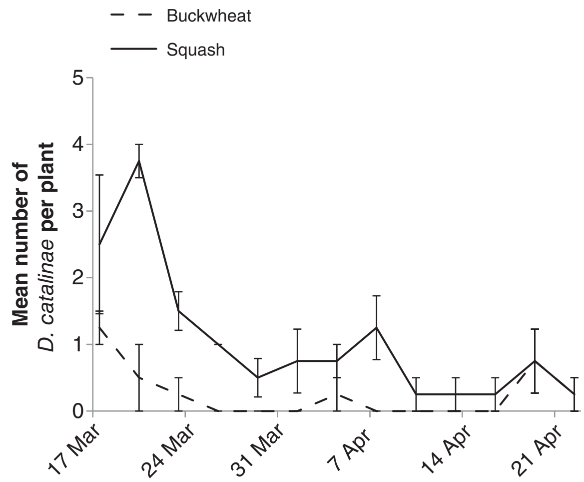

Figure 7. Mean (± SE) number of *D. catalinae* observed for the whitefly preference study in spring 2011.

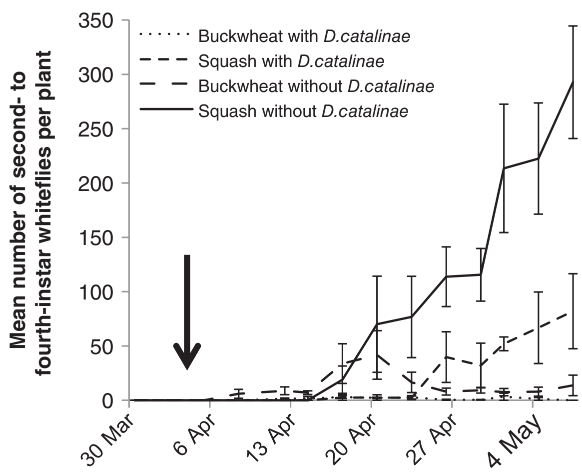

Figure 6. Mean (± SE) number of second- to fourth-instar immature whiteflies observed for the whitefly preference study in spring 2012.[a]

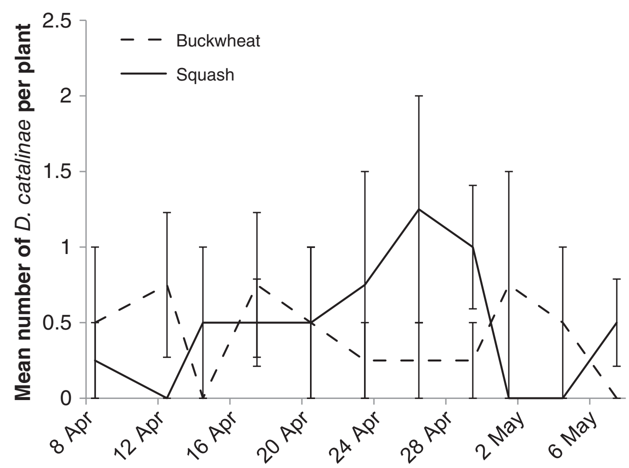

Figure 8. Mean (± SE) number of *D. catalinae* observed for the whitefly preference study in spring 2012.

differences were observed in the last 3 weeks of sampling. There was also a predator × time interaction effect ($F = 1.90$; df = 14, 194; $P = 0.0286$), such that treatment differences were observed in the final 2 weeks of sampling.

In 2012, second- to fourth-instar immature whitefly densities were different between plant species ($F = 68.92$; df = 1, 181; $P \leq 0.0001$) and *D. catalinae* treatments ($F = 44.67$; df = 1, 181; $P \leq 0.0001$), and there was an interaction effect ($F = 22.48$; df = 1, 181; $P \leq 0.0001$). There were more second- to fourth-instar immature whiteflies on zucchini squash than on buckwheat (Fig. 6). Fewer second- to fourth-instar immature whiteflies were recorded on zucchini squash in treatments with *D. catalinae* compared with treatments without *D. catalinae*; however, there was no significant difference in whitefly densities on buckwheat when comparing the *D. catalinae* treatments (Fig. 6). Second- to fourth-instar immature whitefly densities were also different over time ($F = 10.64$; df = 13, 181; $P \leq 0.0001$), and there was a plant species × time interaction effect ($F = 10.41$; df = 13, 181; $P \leq 0.0001$), such that treatment differences were observed in the last 2 weeks of sampling. There was also a predator × time interaction effect ($F = 3.52$; df = 13, 181; $P \leq 0.0001$), such that treatment differences were observed from the third to the sixth week of sampling.

In 2011, *D. catalinae* densities were different between plant species ($F = 36.25$; df = 1, 78; $P \leq 0.0001$) and over time ($F = 7.13$; df = 12, 78; $P \leq 0.0001$), and there was a plant species × time interaction effect ($F = 3.21$; df = 12, 78; $P \leq 0.0001$), such that treatment differences were observed in the first 2 weeks of sampling. More *D. catalinae* were observed on zucchini squash than on buckwheat (Fig. 7). In 2012, *D. catalinae* densities were not significantly different between plant species ($F = 0.14$; df = 1, 66; $P = 0.7081$) or over time ($F = 0.32$; df = 10, 66; $P = 0.9720$), and there was no interaction effect ($F = 1.04$; df = 10, 66; $P = 0.4209$) (Fig. 8).

[a]Arrow indicates when *D. catalinae* was released

4 DISCUSSION

Whitefly densities were higher on zucchini squash than on buckwheat plants. This finding suggests that buckwheat is not an attractive host for *B. tabaci* biotype B, and therefore supports the recommendation by Hooks *et al.*[10] and Frank and Liburd[9] that buckwheat could serve as an important living mulch in cucurbit production systems. The incorporation of buckwheat into the production system can be advantageous because it flowers profusely and harbors beneficial insects. The introduction of *D. catalinae* on zucchini squash reduces whitefly populations owing to its high prey consumption rates of immature whiteflies, as reported by Heinz *et al.*[14] In the absence of *D. catalinae*, as seen in our controlled studies, whitefly populations on squash increased exponentially at the end of the 6 week sampling period. For the first 3 weeks during the sampling period, adult whiteflies were not affected by the presence of *D. catalinae*, which is consistent with observations that the predatory beetle does not feed on the adult whitefly stages. However, towards the end of the sampling period, squash treatments where *D. catalinae* was absent had more adult whiteflies compared with treatments where *D. catalinae* was present. This finding also correlates with a greater density of immature whiteflies on plants where *D. catalinae* was absent. Therefore, more adults were able to emerge from surviving immature stages where *D. catalinae* was absent.

In general, significant differences in whitefly densities were not recorded among the buckwheat treatments. As whitefly populations remained low on buckwheat plants, it was difficult to observe a difference in whitefly densities on buckwheat when *D. catalinae* was introduced. An exception was the significant reduction in second- to fourth-instar immature whitefly densities on buckwheat with *D. catalinae* when compared with whitefly densities on buckwheat without *D. catalinae*. This suggests that *D. catalinae* can also be an efficient predator on buckwheat.

In 2011, more adult *D. catalinae* were found on squash where the whitefly density was greater compared with buckwheat. Heinz *et al.*[14] indicated that, when *D. catalinae* locates its host at high densities, the propensity to disperse is low. In 2012 there was no difference in adult *D. catalinae* distribution between squash and buckwheat over the sampling period. This finding is different from 2011, but could possibly be explained by fewer adult *D. catalinae* being released in 2012 (10 adults cage^{-1}) compared with 2011 (30 adults cage^{-1}), and therefore significant differences in distribution were more difficult to demonstrate. There were also more immature whiteflies present on plants in 2012 compared with 2011, because there were fewer adult *D. catalinae* present in 2012 compared with 2011. This may have influenced *D. catalinae* distribution and could suggest that there may be a saturation limit of the pest that may influence *D. catalinae* dispersal.

In conclusion, *D. catalinae* when used in conjunction with buckwheat as a living mulch could aid in reducing whiteflies on zucchini squash and possibly reduce the incidence of whitefly-transmitted diseases. Future field studies should consider the effect of intercropping buckwheat with zucchini squash on populations of whiteflies and *D. catalinae*. The efficacy of this study in the greenhouse will hopefully support the feasibility of intercropping buckwheat with zucchini squash in the field, releasing *D. catalinae* as a biological control to reduce populations of *B. tabaci* biotype B, and enhance the sustainability of cucurbit production systems.

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