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Intercropping buckwheat with squash to reduce insect pests and disease incidence and increase yield

Janine M. Razze, Oscar E. Liburd, and Susan E. Webb

Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

ABSTRACT

Aphids and whiteflies are significant direct pests of squash and transmit plant viruses. The use of buckwheat, *Fagopyrum esculentum* Moench, as a living mulch intercropped with squash has been shown to reduce insect pests and diseases while increasing the abundance of beneficial insects; however, how to best implement buckwheat in squash fields has not been determined. Several arrangements of intercropping buckwheat and squash were evaluated, with and without the introduction of a natural enemy, *Delphastus catalinae* (Horn), to find a tactic that reduces insect pests and disease incidence while increasing marketable yield. Intercropping treatments included planting strips of buckwheat alternating on either side of the squash with and without *D. catalinae* (arrangement A), planting buckwheat in the middle of squash planted on both sides of the bed with and without *D. catalinae* (arrangement B), buckwheat planted on both sides of squash (arrangement C), and a bare ground treatment. Aphid densities and insect-transmitted viruses were reduced, while natural enemies were more abundant, in buckwheat treatments compared with bare ground treatments. Plant size was reduced in intercropping arrangements B and C compared with arrangement A. Marketable yields were not different between the bare ground treatment and buckwheat arrangements A and B.

KEYWORDS
Aphids; *Bemisia tabaci* biotype B; buckwheat; *Delphastus catalinae*; intercropping; zucchini squash

Zucchini squash, *Cucurbita pepo* L., is a high value crop in Florida and throughout the United States (Nyoike and Liburd 2010). During 2012, Florida harvested 3,925 ha of zucchini squash valued at $67 million USD (Florida Department of Agriculture and Consumer Services 2013). In the southeastern United States, squash is typically produced by methods of direct seeding and plastic mulch (Olson et al. 2012).

Aphids (Hemiptera: Aphididae) and the whitefly *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae) are major pests of zucchini squash. Feeding by whitefly nymphs causes plant physiological disorders (Schuster, Kring, and Price 1991; Yokomi, Hoelmer, and Osborne 1990) and aphid and whitefly adults transmit plant viruses (Akad et al. 2008;

CONTACT Janine M. Razze jrazze@ufl.edu Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, USA.

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Webb, Hiebert, and Kucharek 2003) that are serious problems throughout the southeastern United States. During the fall season, aphids and whiteflies can reach high population densities (Schuster, Polston, and Price 1992) and can transmit and spread viruses very quickly (Jones 2003). Aphids transmit viruses in a nonpersistent manner by feeding on an infected plant and transferring the virus to a healthy plant during the next feeding (Castle et al. 1992). Aphid-transmitted viruses affecting zucchini squash include *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), and *Papaya ringspot virus*-watermelon strain (PRSV-W) (Frank and Liburd 2005; Summers, Mitchell, and Stapleton 2004; Webb, Hiebert, and Kucharek 2003). *Bemisia tabaci* biotype B transmits geminiviruses, particularly *Cucurbit leaf crumple virus* (CuLCrV), in a persistent manner, such that once the virus is acquired by the whitefly it retains the ability to transmit it for a long period of time. Therefore, whitefly transmitted viruses have the potential to cause significant squash yield losses (Akad et al. 2008; Nyoike, Liburd, and Webb 2008).

One of the most damaging plant physiological disorders in squash is squash silverleaf (SSL) disorder, which is associated with the feeding of immature whiteflies (Yokomi, Hoelmer, and Osborne 1990). SSL is characterized by silvering of the upper leaf surface and bleaching of the fruit, which can reduce the quality of the fruit produced depending on the severity of the disorder (Cardoza, McAuslane, and Webb 2000; Costa et al. 1994; McAuslane et al. 2004).

The use of systemic insecticides, such as imidacloprid, can be an important management tactic for suppressing aphid and whitefly populations on squash, hindering the spread of viruses within the field (Nyoike and Liburd 2010). However, sustainable management of aphids and whiteflies with insecticides can be problematic for several reasons. Whiteflies and particularly aphids can acquire and transmit viruses quickly (Summers, Mitchell, and Stapleton 2004), and these insects have also become resistant to a number of insecticides (Foster, Denholm, and Thompson 2002; Nauen, Stumpf, and Elbert 2002). Furthermore, many insecticides can have detrimental effects on pollinators, which are essential for the production of squash (Blacquière et al. 2012). There is a need to develop integrated pest management (IPM) strategies that are compatible with the principles for organic production for growers interested in sustainable agriculture.

Several studies evaluating the efficacy of buckwheat, *Fagopyrum esculentum* Moench, as a living mulch in squash production systems for the control of aphids and whiteflies have demonstrated successful pest suppression, as well as lower incidences of insect-transmitted viruses (Frank and Liburd 2005; Hooks, Valenzuela, and Defrank 1998; Nyoike and Liburd 2010). In addition, flowering buckwheat attracts beneficial insects to the cucurbit crop (Frank and Liburd 2005). Naturally occurring beneficial predators and parasitoids in the field serve as an important control tactic for managing aphid and whitefly populations.

The coccinellid beetle *Delphastus catalinae* (Horn), a specialist predator feeding only on whiteflies, is a good biological control candidate for whiteflies as a result of high prey consumption rates, longevity, and high fecundity rates (Heinz et al. 1999), and is commercially available for whitefly control (Simmons, Legaspi, and Legaspi 2008). Razze, Liburd, and McSorley (2015) reported a significant reduction in immature whitefly populations when *D. catalinae* adults were released on squash compared with squash plants where *D. catalinae* was not released in a greenhouse experiment. Heinz et al. (1999) also demonstrated that adult beetles released from a central point source dispersed less than 1 m/d at high prey densities (greater than 50 whitefly immatures per cm^2 of leaf area). This low propensity to disperse suggests that movement of *D. catalinae* out of experimental plots should be minimal.

Although buckwheat has been used successfully in squash to reduce populations of pests and the spread of viruses in the field, there is no information on how buckwheat should be deployed in a squash production system to improve pest management and specifically crop yield, which can be significantly reduced as a result of early season competition for shared resources (Nyoike and Liburd 2010). Adjustments in plant spacing and time of planting may increase marketable yields (Nyoike and Liburd 2010), but more research is needed in this area.

The purpose of this study was to evaluate several methods of intercropping buckwheat and squash to develop a system that reduces pests and disease incidence while increasing marketable yield. The specific objectives were: a) to compare several tactics of intercropping buckwheat and squash and their effects on pest and natural enemy densities, disease incidence, and marketable yields in field grown squash; b) to incorporate a key natural enemy, *D. catalinae*, into buckwheat and squash crops to determine the effects on pest populations and marketable yields; and c) to use an on-farm demonstration model to implement the buckwheat-squash intercropping tactic and to introduce *D. catalinae* into a grower's field.

Materials and methods

Plot preparation and experimental design

Comparing tactics for intercropping buckwheat into squash production system while incorporating D. catalinae

The study was conducted during the fall of 2011 and 2012 at the University of Florida Plant Science Research and Education Unit (PSREU) at Citra, FL. The experimental design consisted of a randomized complete block with four replicates. Each plot contained 4 rows and measured 7.6 m × 7.6 m. Plots were separated by 4.5 m of bare soil on all sides. Planting beds were raised 30 cm and each bed received two drip irrigation lines. Zucchini squash variety Cashflow (Johnny's Selected Seeds, Winslow, ME) was hand seeded on September 8, 2011 and September 10, 2012. Plant spacing was approximately 30.5 cm between squash plants. Missing plants were replaced after germination using squash transplants that were previously established in the greenhouse. Buckwheat (Johnny's Selected Seeds, Winslow, ME) was hand seeded 10 d and 3 d before planting the squash seeds in 2011 and 2012, respectively. In 2011, greater competition between buckwheat and the squash was observed; therefore, the planting days were reduced from 10 d to 3 d prior to planting squash in 2012. In 2011, buckwheat seeds were sown by hand in a continuous line along the length of the bed. As a result, buckwheat density was high in 2011 and buckwheat plants had to be thinned out to reduce competition between buckwheat and squash. In 2012, buckwheat seeds were sown by hand every 1.27 cm.

In 2011, the treatments included the following planting arrangements: a) "buckwheat alternating," where 2.5 m strips of buckwheat and bare ground were alternated on either side of squash planted in the middle of the bed; b) "buckwheat alternating with *D. catalinae*," identical to the first treatment, but *D. catalinae* was released into the plot; c) "buckwheat in the middle," where buckwheat was planted in the middle of the bed with squash planted on both sides; d) "buckwheat in the middle with *D. catalinae*," identical to the third treatment, but *D. catalinae* was released into the plot; and e) "buckwheat on both sides" served as a positive control with solid rows of buckwheat growing on both sides of the squash (Figure 1). This was a treatment that was previously evaluated in Nyoike and Liburd (2010), where yield was lower in squash due to competition from buckwheat. Buckwheat alternating treatments were referred to as buckwheat A; buckwheat middle treatments were

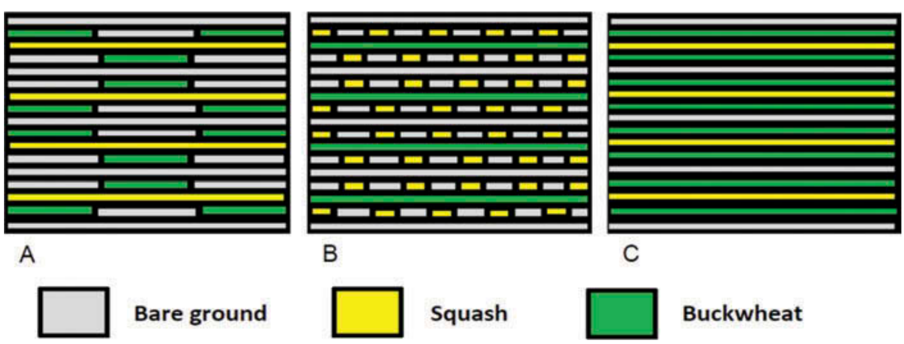

Figure 1. Diagrams of the different buckwheat arrangements implemented in the intercropping field study. A) Buckwheat A, where buckwheat is planted as alternating strips on either side of the squash; B) buckwheat B, where buckwheat is planted in the middle of the squash planted on both sides of the bed; and C) buckwheat C, where buckwheat is planted continuously on both sides of the squash.

referred to as buckwheat B; and the buckwheat both sides treatment was referred to as buckwheat C. The same arrangements were used in 2012; however, a sixth treatment was added, bare ground, which served as a negative control with squash planted in the middle of the bed with no buckwheat in the plot.

In treatments with *D. catalinae*, 100 adults were released into each plot approximately 2.5 wk after the squash was planted to ensure that adult whiteflies had sufficient time to colonize and reproduce. *Delphastus catalinae* adults were originally purchased from Biocontrol Network, LLC (Brentwood, TN, USA) and maintained on a colony of *B. tabaci* biotype B infesting cotton and collards in the Small Fruit and Vegetable IPM Laboratory, Department of Entomology and Nematology, University of Florida, Gainesville, FL. The colony was maintained at 28°C with 70 ± 5% RH on L:D 14:10.

On-farm demonstration to intercrop buckwheat into squash production system while incorporating D. catalinae

This study was conducted during the fall of 2013 at an organic farm in north-central Florida, as a completely randomized design with three treatments and three replicates. Each plot contained 4 rows and measured 4 m × 4 m. Plots were separated by 4.5 m of bare soil on all sides. Planting beds were raised 30 cm and each bed received two drip irrigation lines. Squash was hand seeded approximately 30.5 cm apart on September 6, 2013. Buckwheat (Johnny's Selected Seeds, Winslow, ME) was hand seeded 2 d before planting the squash. Buckwheat seeds were planted approximately 1.27 cm apart.

The following planting arrangements were evaluated: a) buckwheat A, where 1.3 m strips of buckwheat and bare ground were alternating on either side of summer squash; b) buckwheat B, where buckwheat was planted in the middle of the bed with squash planted on both sides; and c) "mixed varieties," the grower's standard treatment (control), with three different varieties of squash randomly mixed and planted on both sides of the bed and otherwise surrounded by bare ground with no buckwheat planted. The yellow summer squash variety Zephyr (Johnny's Selected Seeds, Winslow, ME) was used in the first two treatments. The third treatment was a grower standard consisting of three mixed squash varieties (Johnny's Selected Seeds, Winslow, ME): Sunburst patty pan summer squash, Eight Ball zucchini squash, and One Ball zucchini squash. After germination, missing plants were replaced using squash transplants that were previously established in the greenhouse.

Fifty adult *D. catalinae* were obtained from the colony previously described and released in plots with buckwheat approximately 2.5 wk after planting squash to ensure that adult whiteflies had sufficient time to colonize and reproduce.

Agronomic practices for organic squash production in all 3 yr were adapted from the standard production guide for squash in North Florida

(Olson et al. 2012). Dipel DF (Valent BioSciences Corporation, Libertyville, IL, USA) and Entrust (Dow Agrosciences LLC, Indianapolis, IN, USA) were rotated and applied during the growing season for the control of melonworm, *Diaphania hyalinata* Linnaeus, and pickleworm, *Diaphania nitidalis* (Stoll) (Lepidoptera: Pyralidae). The fungicides Serenade Max (AgraQuest Inc., Davis, CA, USA) and Regalia (Marrone Bio Innovations, Davis, CA) were sprayed as needed to control powdery mildew. A blended dry granular fertilizer compliant with organic systems [Nature Safe (10–2-8) (Griffin Industries LLC, Cold Spring, KY, USA)] was incorporated into the soil at planting and followed by a second application of the same fertilizer four weeks after planting. Weeds were managed mechanically throughout the growing season.

Sampling

Alate and apterous aphids, adult and immature whiteflies, disease incidence, natural enemies, plant size, and marketable yield in each plot were monitored on squash plants and recorded as described below.

Aphids

Alate and apterous aphids, both immatures and adults, were sampled weekly from randomly selected plants in the outer rows of each plot using the leaf-turn method as detailed in Nyoike and Liburd (2010). In 2011 and 2012, nine randomly chosen plants were selected and, in 2013, five randomly chosen plants were selected. Alate aphids were also monitored using blue-colored pan traps (Solo, Lake Forest, IL) that were secured within tomato cages and contained approximately 250 cm^3 of 5% detergent solution (Colgate-Palmolive Co., New York, NY, USA). Two traps were placed in each plot at opposing corners in 2011 and 2012, and one trap was placed in each plot in 2013. The contents of the traps were collected weekly and taken back to the laboratory where the number of alate aphids was counted and recorded.

Whiteflies

Adult whiteflies were monitored using yellow sticky Pherocon AM unbaited traps (Great Lakes IPM, Vestaburg, MI, USA) that were mounted on wooden stakes and placed just above the plant canopy. Two traps were placed in the inner rows of each plot at opposing ends in 2011 and 2012, and one trap was placed in the inner rows of each plot in 2013. Traps were left in the field for 24 h, after which the number of adult whiteflies per trap was counted at the laboratory. To count immature whiteflies, three of the nine leaves used for sampling apterous aphids in 2011 and 2012 and two of the five leaves used for sampling apterous aphids in 2013 were excised and brought back to the laboratory. A 3.14-cm^2 leaf disc was taken from each leaf using a cork borer.

Whitefly nymphs were counted using a dissecting microscope and the total number of all immature stages was recorded.

Diseases

Visual observations of viral symptoms and incidence were monitored each week by recording the number of plants in each plot showing virus symptoms. Leaves were collected and assayed for the most commonly occurring aphid-transmitted cucurbit viruses by a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams 1977). All antisera were obtained from Agdia, Inc. Elkhart, IN. Leaves were also assayed using polymerase chain reaction (PCR) to confirm the presence of the whitefly-transmitted *Cucurbit leaf crumple virus* (CuLCrV), as described in Akad et al. (2008). Squash silverleaf (SSL) was also monitored each week. Ten plants were randomly selected from the inner rows of each plot in 2011 and 2012, and five plants were randomly selected from the inner rows of each plot in 2013. Plants were scored with an arbitrary scale adapted from Yokomi, Hoelmer, and Osborne (1990) that ranges from 0, which indicates a healthy plant, to 5, which indicates a plant with all leaves completely silvered.

Natural enemies

Natural enemies were monitored each week using in situ counts. Six plants were randomly selected from the outside rows of each plot in 2011 and 2012, and three plants were randomly selected from the outside rows of each plot in 2013. The numbers of predators and parasitoids on each plant were counted and recorded. Natural enemies were also monitored using pitfall traps containing a 5% detergent solution (Colgate-Palmolive Co., New York, NY) and the yellow sticky trap used to monitor adult whiteflies. Two pitfall traps were placed in the inner rows of each plot in opposing corners in 2011 and 2012, and one trap was placed in the inner rows in 2013. Yellow sticky traps were left in the field for 24 h and pitfall traps were left in the field for 1 wk. Traps were collected weekly and taken back to the laboratory where natural enemies were identified and recorded.

Plant measurements and marketable yields

Squash plant height and width were measured each week from ten randomly selected plants in the inner rows of each plot in 2011 and 2012, and five randomly selected plants in 2013, as described in Frank and Liburd (2005). Plant height was measured from the ground to the terminal bud with a tape measure. Plant width was measured along the length between the two widest opposing lateral shoots. Squash was harvested from the inner rows of each plot. Marketable fruit was harvested and weighed in the field every other day until the end of the season in early November. Fruit was determined to be marketable by examining the fruit for evidence of viral symptoms or physiological disorders, such as irregular fruit ripening, as well as pest damage from pickleworm and wet rot of fruits caused by a fungus.

Data analysis

Aphid, whitefly, natural enemy, disease incidence, and plant measurement data were separated by year and analyzed by a repeated measures analysis of variance procedure (ANOVA; PROC GLM, SAS Institute 2009). The model was constructed to examine treatment, time, and the interaction treatment × time as the fixed effects, and block was designated as a random factor. Marketable yield data were summed over the entire growing season per year and analyzed using ANOVA (PROC GLM, SAS Institute 2009). Within the model, the following preplanned orthogonal contrasts were conducted: buckwheat A versus buckwheat B, buckwheat A versus buckwheat C, buckwheat B versus buckwheat C, buckwheat A versus bare ground, buckwheat B versus bare ground, buckwheat C versus bare ground, buckwheat A versus mixed varieties, and buckwheat B versus mixed varieties. Treatments with identical buckwheat arrangements were also compared to evaluate the influence of released *D. catalinae* on pest populations and yields. Aphid, whitefly, and natural enemy counts were square-root transformed, and disease incidence and marketable yield data were log transformed to stabilize variances. Reported means are from non-transformed data. Treatment means were separated by least significant differences (LSD) test (SAS Institute 2009) where ANOVA indicated a significant effect on the model by factor, and differences among treatments were considered significant if $P \leq 0.05$.

Results

Aphids

Several aphid species were recorded during the study, but the two most common are the melon aphid (*Aphis gossypii* Glover) occurring about 60% of the time and the green peach aphid, *Myzus persicae* (Sulzer) occurring about 30%. Other species encountered include the cowpea aphid, *Aphis craccivora* Koch and *Macrosiphum* spp. The majority of aphids caught in the pan traps were winged aphids, but the taxonomic distribution was similar to those observed on the plant.

In the studies conducted at the PSREU in 2011, the number of aphids sampled by in situ counts differed over time ($F = 73.79$; $df = 4, 830$; $P \leq 0.0001$), with no treatment ($F = 1.84$; $df = 4, 830$; $P = 0.1196$) or interaction effect ($F = 1.28$; $df = 16, 830$; $P = 0.2034$). The number of aphids in pan traps differed by treatment ($F = 2.80$; $df = 4, 165$; $P = 0.0279$) and over time ($F = 20.02$; $df = 4, 165$; $P \leq 0.0001$), with no interaction effect ($F = 1.54$; $df = 16, 165$; $P = 0.0900$). There were more aphids in buckwheat B compared with buckwheat A ($F = 9.36$; $df = 1, 165$; $P = 0.0026$) and buckwheat C ($F = 5.20$; $df = 1, 165$; $P = 0.0239$) (Figure 2).

In 2012, the number of aphids sampled by in situ counts differed by treatment ($F = 3.00$; $df = 5, 1050$; $P = 0.0107$) and over time ($F = 5.98$; $df = 4, 1050$; $P \leq 0.0001$), with no interaction effect ($F = 1.30$; $df = 20, 1050$; $P = 0.1700$). There were fewer aphids on squash plants in buckwheat C compared with buckwheat A ($F = 5.42$; $df = 1, 1050$; $P = 0.0201$), buckwheat B ($F = 7.15$; $df = 1, 1050$; $P = 0.0076$), and the bare ground treatment ($F = 13.91$; $df = 1, 1050$; $P = 0.0002$) (Figure 2). There were also fewer aphids on squash plants in buckwheat A compared with the bare ground treatment ($F = 3.92$; $df = 1, 1050$; $P = 0.0481$) (Figure 2). The number of aphids in pan traps differed over time ($F = 5.22$; $df = 4, 210$; $P = 0.0005$), with no treatment ($F = 0.52$; $df = 5, 210$; $P = 0.7605$) or interaction effect ($F = 0.87$; $df = 20, 210$; $P = 0.6286$).

In the on-farm study (2013), the number of aphids sampled by in situ counts differed by treatment ($F = 5.25$; $df = 2, 210$; $P = 0.0060$), over time ($F = 5.54$;

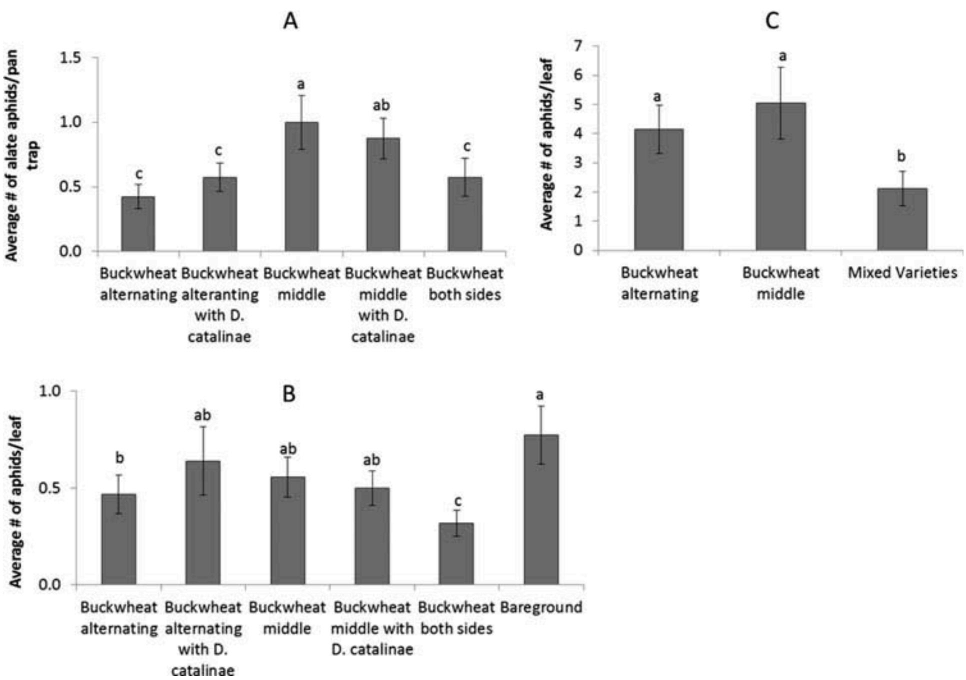

Figure 2. Mean (±*SE*) number of aphids sampled per squash over a five-week period for the intercropping field study in (A) 2011, (B) 2012, and (C) 2013. Aphids sampled by in situ counts were not significantly different by treatment in 2011, and (A) is the number of alate aphids collected from pan traps. Alate aphids collected from traps were not significantly different by treatment in 2012 and 2013, and (B) and (C) are the numbers of aphids sampled by in situ counts. Treatments with the same letter are not significantly different ($P \leq 0.05$).

df = 4, 210; *P* = 0.0003), and there was an interaction effect (*F* = 3.31; *df* = 8, 210; *P* = 0.0014) such that there were treatment differences in the fourth and fifth weeks of sampling. There were fewer aphids on squash plants in the mixed varieties treatment compared with buckwheat A (*F* = 6.73; *df* = 1, 210; *P* = 0.0102) and buckwheat B (*F* = 8.87; *df* = 1, 210; *P* = 0.0032) (Figure 2). The number of aphids in pan traps were not significantly different by treatment (*F* = 0.24; *df* = 2, 30; *P* = 0.7844), over time (*F* = 1.68; *df* = 4, 30; *P* = 0.1805), and there was no interaction effect (*F* = 0.36; *df* = 8, 30; *P* = 0.9346).

Whiteflies

In 2011, immature whitefly counts from leaf-disc assays differed by treatment (*F* = 3.04; *df* = 4, 260; *P* = 0.0180), over time (*F* = 74.62; *df* = 4, 260; *P* ≤ 0.0001), and there was an interaction effect (*F* = 1.72; *df* = 16, 260; *P* = 0.0439) such that there were treatment differences the first week of sampling. There were fewer immature whiteflies on squash plants in buckwheat A compared with buckwheat C (*F* = 11.69; *df* = 1, 260; *P* = 0.0007) (Figure 3). The number of adult whiteflies on yellow sticky traps differed over time (*F* = 72.83; *df* = 4, 165; *P* ≤ 0.0001) but there was no treatment (*F* = 1.58; *df* = 4, 165; *P* = 0.1826) or interaction effect (*F* = 0.56; *df* = 16, 165; *P* = 0.9086).

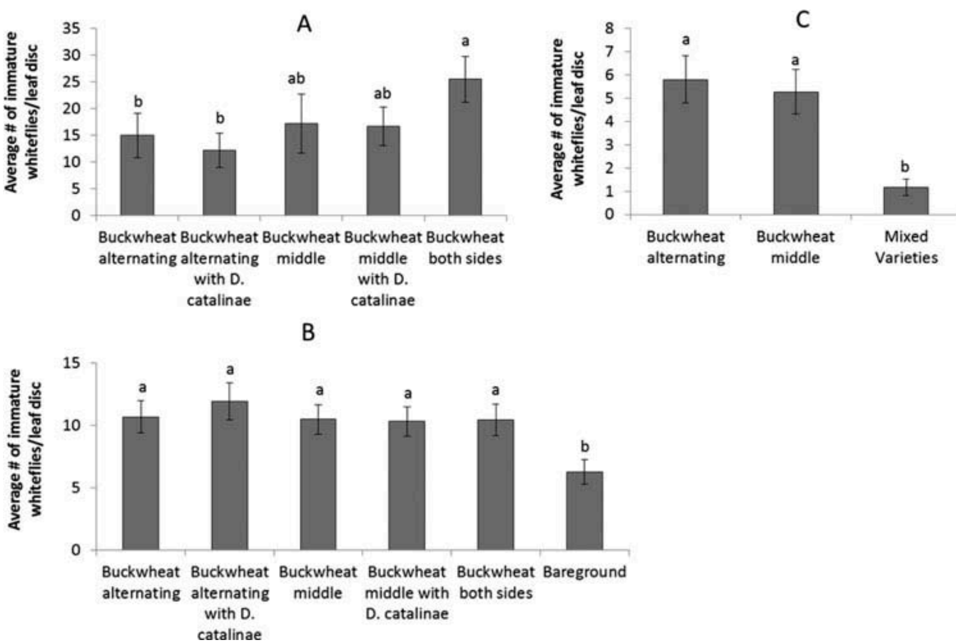

Figure 3. Mean (±*SE*) number of immature whiteflies sampled from 3.14-cm^2 leaf discs over a five-week period for the intercropping field study in (A) 2011, (B) 2012, and (C) 2013. Treatments with the same letter are not significantly different (*P* ≤ 0.05).

In 2012, immature whitefly counts from leaf-disc assays differed by treatment ($F = 3.50$; $df = 5, 330$; $P = 0.0042$), over time ($F = 20.02$; $df = 4, 330$; $P \leq 0.0001$), and there was an interaction effect ($F = 1.79$; $df = 20, 330$; $P = 0.0202$), such that there were treatment differences the first, third, and fourth weeks of sampling. There were fewer immature whiteflies on squash plants in the bare ground treatment compared with treatments with buckwheat, including buckwheat A ($F = 14.59$; $df = 1, 330$; $P = 0.0002$), buckwheat B ($F = 12.79$; $df = 1, 330$; $P = 0.0004$), and buckwheat C ($F = 7.54$; $df = 1, 330$; $P = 0.0064$) (Figure 3). The number of adult whiteflies on yellow sticky traps differed over time ($F = 90.14$; $df = 5, 252$; $P \leq 0.0001$) but there was no treatment effect ($F = 1.76$; $df = 5, 252$; $P = 0.1219$).

In 2013, immature whitefly counts from leaf-disc assays in the on-farm demonstration differed by treatment ($F = 19.02$; $df = 2, 75$; $P \leq 0.0001$), over time ($F = 2.73$; $df = 4, 75$; $P = 0.0350$), and there was an interaction effect ($F = 2.60$; $df = 8, 75$; $P = 0.0144$) such that there were treatment differences in all but the second week of sampling. There were fewer immature whiteflies on squash plants in the mixed varieties treatment compared with buckwheat A ($F = 31.23$; $df = 1, 75$; $P \leq 0.0001$) and buckwheat B ($F = 25.54$; $df = 1, 75$; $P \leq 0.0001$) (Figure 3). The number of adult whiteflies on yellow sticky traps differed over time ($F = 6.85$; $df = 4, 30$; $P = 0.0005$), but there was no treatment ($F = 2.28$; $df = 2, 30$; $P = 0.1201$) or interaction effect ($F = 1.39$; $df = 8, 30$; $P = 0.2420$).

Diseases

Samples obtained from the field and tested for viruses were predominantly found to test positive for *Cucurbit leaf crumple virus* using PCR in all three years. Although not prevalent in the field, several leaf samples tested positive for *Zucchini yellow mosaic virus* and *Papaya ringspot virus* watermelon strain using ELISA techniques in 2011 and 2012, but not in 2013 at the on-farm demonstration trial.

In 2011, virus incidence differed over time ($F = 12.35$; $df = 4, 70$; $P \leq 0.0001$) but there was no treatment ($F = 1.08$; $df = 4, 70$; $P = 0.3710$) or interaction effect ($F = 0.71$; $df = 16, 70$; $P = 0.7785$) (Figure 4). Alternatively, SSL ratings differed by treatment ($F = 2.51$; $df = 4, 925$; $P = 0.0407$) and over time ($F = 258.68$; $df = 4, 925$; $P \leq 0.0001$), with no interaction effect ($F = 0.68$; $df = 16, 925$; $P = 0.8123$). SSL ratings were greater in buckwheat C compared with buckwheat A ($F = 8.18$; $df = 1, 925$; $P = 0.0043$) and buckwheat B ($F = 6.04$; $df = 1, 925$; $P = 0.0142$) (Figure 5).

In 2012, virus incidence differed by treatment ($F = 8.07$; $df = 5, 90$; $P \leq 0.0001$) and over time ($F = 15.39$; $df = 4, 90$; $P \leq 0.0001$), with no interaction effect ($F = 0.62$; $df = 20, 90$; $P = 0.8873$). There were more plants with virus symptoms in the bare ground treatment compared with treatments with buckwheat, including buckwheat A ($F = 37.17$; $df = 1, 90$; $P \leq 0.0001$), buckwheat B ($F = 9.02$; $df = 1, 90$; $P = 0.0034$), and buckwheat

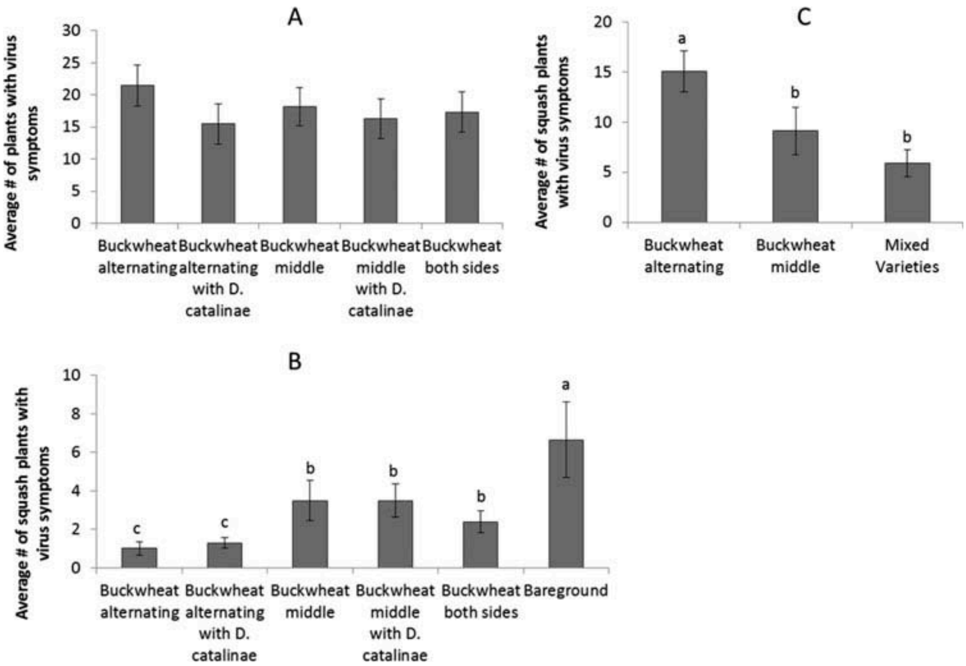

Figure 4. Mean (±*SE*) number of squash plants with virus symptoms over a five-week period for the intercropping field study in (A) 2011, (B) 2012, and (C) 2013. Treatments with the same letter are not significantly different ($P \leq 0.05$).

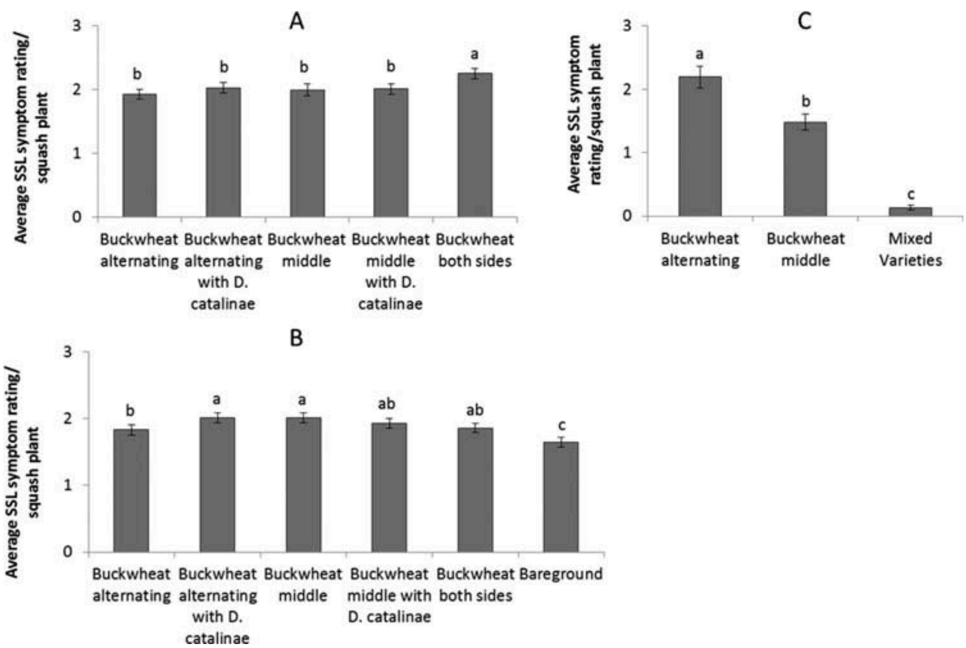

Figure 5. Mean (±*SE*) squash silverleaf (SSL) disorder symptom rating per squash plant over a five-week period for the intercropping field study in (A) 2011, (B) 2012, and (C) 2013. Treatments with the same letter are not significantly different ($P \leq 0.05$).

C ($F = 11.58$; $df = 1, 90$; $P = 0.0010$) (Figure 4). There were also fewer plants with virus symptoms in buckwheat A compared with plants in buckwheat B ($F = 14.35$; $df = 1, 90$; $P = 0.0003$) and buckwheat C ($F = 4.70$; $df = 1, 90$; $P = 0.0329$) (Figure 4). SSL ratings differed by treatment ($F = 4.96$; $df = 5, 1170$; $P = 0.0002$), over time ($F = 221.93$; $df = 4, 1170$; $P \leq 0.0001$), and there was an interaction effect ($F = 2.85$; $df = 20, 1170$; $P \leq 0.0001$) such that there were treatment differences the first, second, fourth, and fifth weeks of sampling. SSL ratings were lower in the bare ground treatment compared with treatments with buckwheat, including buckwheat A ($F = 13.12$; $df = 1, 1170$; $P = 0.0003$), buckwheat B ($F = 18.27$; $df = 1, 1170$; $P \leq 0.0001$), and buckwheat C ($F = 5.91$; $df = 1, 1170$; $P = 0.0152$) (Figure 5).

In 2013, virus incidence differed by treatment ($F = 7.85$; $df = 2, 30$; $P = 0.0018$) and over time ($F = 7.73$; $df = 4, 30$; $P = 0.0002$), with no interaction effect ($F = 0.29$; $df = 8, 30$; $P = 0.9636$). There were more plants with virus symptoms in buckwheat A compared with buckwheat B ($F = 7.43$; $df = 1, 30$; $P = 0.0106$) and the mixed varieties treatment ($F = 14.84$; $df = 1, 30$; $P = 0.0006$) (Figure 4). SSL ratings differed by treatment ($F = 127.72$; $df = 2, 210$; $P \leq 0.0001$), over time ($F = 18.38$; $df = 4, 210$; $P \leq 0.0001$), and there was an interaction effect ($F = 5.27$; $df = 8, 210$; $P \leq 0.0001$) such that there were treatment differences in all weeks sampled. SSL ratings were lower in the mixed varieties treatment compared with buckwheat A ($F = 234.97$; $df = 1, 210$; $P \leq 0.0001$) and buckwheat B ($F = 134.13$; $df = 1, 210$; $P \leq 0.0001$) (Figure 5). SSL ratings were also lower in buckwheat B compared with buckwheat A ($F = 14.04$; $df = 1, 210$; $P = 0.0002$) (Figure 5).

Natural enemies

The natural enemies observed by in situ counts for all three years included green lacewings (Neuroptera: Chrysopidae); lady beetles (Coleoptera: Coccinellidae); ground beetles (Coleoptera: Carabidae); hover flies (Diptera: Syrphidae); big-eyed bugs, *Geocoris* spp. (Hemiptera: Lygaeidae); minute pirate bugs, *Orius* spp. (Hemiptera: Anthocoridae); and spiders (Araneae). In 2011, lacewings counts differed by treatment ($F = 6.90$; $df = 4, 355$; $P \leq 0.0001$) and over time ($F = 13.54$; $df = 4, 355$; $P \leq 0.0001$), but there was no interaction effect ($F = 1.09$; $df = 16, 355$; $P = 0.3634$). There were more lacewings on squash plants in buckwheat A compared with buckwheat B ($F = 4.46$; $df = 1, 355$; $P = 0.0353$) and buckwheat C ($F = 4.30$; $df = 1, 355$; $P = 0.0388$) (Table 1). There were also more lacewings in treatments where *D. catalinae* was not released compared with treatments where *D. catalinae* was released ($F = 12.93$; $df = 1, 355$; $P = 0.0004$) (Table 1).

In 2011, *Orius* counts differed by treatment ($F = 4.96$; $df = 4, 355$; $P = 0.0007$), over time ($F = 17.83$; $df = 4, 355$; $P \leq 0.0001$), and there was an interaction effect ($F = 2.79$; $df = 16, 355$; $P = 0.0003$), such that there were

Table 1. Mean ±*SEM* of natural enemies sampled by in situ counts over a five-week period for the intercropping field study in fall 2011 for five treatments: buckwheat alternating (A), buckwheat alternating (A) with *D. catalinae*, buckwheat in the middle (B), buckwheat in the middle (B) with *D. catalinae*, and buckwheat on both sides (C).

	Mean number of natural enemies per treatment ±*SEM*				
	Buckwheat alternating	Buckwheat alternating with *D. catalinae*	Buckwheat middle	Buckwheat middle with *D. catalinae*	Buckwheat both sides
Chrysopidae[a]	0.69 ± 0.1a	0.13 ± 0.03c	0.29 ± 0.06b	0.17 ± 0.04c	0.2 ± 0.05bc
Coccinellidae[b]	0.05 ± 0.03a	0.11 ± 0.02a	0.09 ± 0.04a	0.04 ± 0.03a	0.08 ± 0.03a
Geocoris spp.[c]	0.11 ± 0.03a	0.09 ± 0.05a	0.04 ± 0.03a	0.09 ± 0.03a	0.1 ± 0.07a
Orius spp.[d]	0.15 ± 0.04c	0.17 ± 0.04c	0.16 ± 0.03c	0.28 ± 0.04b	0.46 ± 0.06a
Araneae[e]	0.16 ± 0.04ab	0.17 ± 0.03a	0.23 ± 0.05a	0.11 ± 0.04ab	0.08 ± 0.03b

Means in rows followed by the same letter are not significantly different ($P \leq 0.05$).
[a]$F = 6.90$; df = 4, 355; $P \leq 0.0001$.
[b]$F = 0.37$; df = 4, 355; $P = 0.8324$.
[c]$F = 0.16$; df = 4, 355; $P = 0.9575$.
[d]$F = 4.96$; df = 4, 355; $P = 0.0007$.
[e]$F = 1.38$; df = 4, 355; $P = 0.2414$.

treatment differences the second week of sampling. There were more *Orius* on squash plants in buckwheat C compared with both buckwheat A ($F = 18.29$; $df = 1, 355$; $P \leq 0.0001$) and buckwheat B ($F = 10.53$; $df = 1, 355$; $P = 0.0013$) (Table 1).

In 2012, lacewings counts differed by treatment ($F = 3.79$; $df = 5, 690$; $P = 0.0021$) and over time ($F = 31.99$; $df = 4, 690$; $P \leq 0.0001$), with no interaction effect ($F = 1.57$; $df = 20, 690$; $P = 0.0530$), such that there were treatment differences the third and fourth weeks of sampling. There were fewer lacewings on squash plants in the bare ground treatment compared with buckwheat B ($F = 6.08$; $df = 1, 690$; $P = 0.0139$) (Table 2). There were also fewer lacewings on squash plants in buckwheat C compared with buckwheat A ($F = 8.80$; $df = 1, 690$; $P = 0.0031$) and buckwheat B ($F = 13.87$; $df = 1, 690$; $P = 0.0002$) (Table 2).

In 2012, *Geocoris* counts differed by treatment ($F = 8.66$; $df = 5, 690$; $P \leq 0.0001$), over time ($F = 87.41$; $df = 4, 690$; $P \leq 0.0001$), and there was an interaction effect ($F = 6.92$; $df = 20, 690$; $P \leq 0.0001$), such that there were treatment differences the fourth and fifth weeks of sampling. There were fewer *Geocoris* on squash plants in the bare ground control compared with all buckwheat treatments, including buckwheat A ($F = 4.23$; $df = 1, 690$; $P = 0.0400$), buckwheat B ($F = 29.40$; $df = 1, 690$; $P \leq 0.0001$), and buckwheat C ($F = 18.46$; $df = 1, 690$; $P \leq 0.0001$) (Table 2). There were also fewer *Geocoris* on squash plants in buckwheat A compared with buckwheat B ($F = 16.98$; $df = 1, 690$; $P \leq 0.0001$) and buckwheat C ($F = 8.43$; $df = 1, 690$; $P = 0.0038$) (Table 2).

Table 2. Mean ±*SEM* of natural enemies sampled by in situ counts for the intercropping field study in fall 2012 for six treatments: buckwheat alternating (A), buckwheat alternating (A) with *D. catalinae*, buckwheat in the middle (B), buckwheat in the middle (B) with *D. catalinae*, buckwheat on both sides (C), and bare ground.

	Mean number of natural enemies per treatment ± SEM					
	Buckwheat alternating	Buckwheat alternating with *D. catalinae*	Buckwheat middle	Buckwheat middle with *D. catalinae*	Buckwheat both sides	Bare ground
Chrysopidae[a]	0.6 ± 0.14a	0.45 ± 0.1ab	0.63 ± 0.1a	0.67 ± .17a	0.22 ± 0.06c	0.32 ± 0.06bc
Geocoris spp.[b]	0.08 ± 0.03 cd	0.17 ± 0.05bc	0.27 ± 0.07ab	0.36 ± 0.09a	0.28 ± 0.07ab	0.03 ± 0.01d
Araneae[c]	0.1 ± 0.03a	0.09 ± 0.03ab	0.08 ± 0.03ab	0.04 ± 0.02b	0.12 ± 0.03a	0.14 ± 0.03a

Means in rows followed by the same letter are not significantly different ($P \leq 0.05$).

[a] $F = 3.79$; df = 5, 690; $P = 0.0021$.

[b] $F = 8.66$; df = 5, 690; $P \leq 0.0001$.

[c] $F = 1.40$; df = 5, 690; $P = 0.2209$.

In the on-farm demonstration plot (2013), there were no significant differences in the mean number of natural enemy taxa observed during in situ counts among treatments.

The natural enemies collected from yellow sticky traps included lady beetles (Coleoptera: Coccinellidae); minute pirate bugs, *Orius* spp. (Hemiptera: Anthocoridae); hover flies (Diptera: Syrphidae); and several parasitoids including *Aphelinus* spp. (Hymenoptera: Aphelinidae); *Encarsia* spp. (Hymenoptera: Aphelinidae); *Eretmocerus* spp. (Hymenoptera: Aphelinidae); and *Trichogramma* spp. (Hymenoptera: Trichogrammatidae).

In 2011, *Trichogramma* counts differed by treatment ($F = 2.80$; $df = 4, 165$; $P = 0.0279$) and over time ($F = 49.82$; $df = 4, 165$; $P \leq 0.0001$), with no interaction effect ($F = 1.24$; $df = 16, 165$; $P = 0.2395$). There were more *Trichogramma* in buckwheat A compared with buckwheat B ($F = 4.66$; $df = 1, 165$; $P = 0.0324$) and buckwheat C ($F = 7.27$; $df = 1, 165$; $P = 0.0078$) (Table 3).

In 2011, *Eretmocerus* counts differed by treatment ($F = 3.23$; $df = 4, 165$; $P = 0.0140$) and over time ($F = 34.93$; $df = 4, 165$; $P \leq 0.0001$), with no interaction effect ($F = 1.07$; $df = 16, 165$; $P = 0.3861$). There were fewer *Eretmocerus* in buckwheat B compared with buckwheat A ($F = 10.93$; $df = 1, 165$; $P = 0.0012$) and buckwheat C ($F = 5.99$; $df = 1, 165$; $P = 0.0154$) (Table 3).

In 2012, *Trichogramma* counts differed by treatment ($F = 11.31$; $df = 5, 252$; $P \leq 0.0001$), over time ($F = 44.47$; $df = 5, 252$; $P \leq 0.0001$), and there was an interaction effect ($F = 2.40$; $df = 25, 252$; $P = 0.0003$), such that there were treatment differences in all sampling weeks except the first week. There were more *Trichogramma* in the bare ground control compared with all buckwheat treatments, including buckwheat A ($F = 33.23$; $df = 1, 252$; $P \leq 0.0001$),

Table 3. Mean ±*SEM* of natural enemies collected from yellow stick traps over a five-week period for the intercropping field study in fall 2011 for five treatments: buckwheat alternating (A), buckwheat alternating (A) with *D. catalinae*, buckwheat in the middle (B), buckwheat in the middle (B) with *D. catalinae*, and buckwheat on both sides (C).

	Mean number of natural enemies per treatment ±*SEM*				
	Buckwheat alternating	Buckwheat alternating with *D. catalinae*	Buckwheat middle	Buckwheat middle with *D. catalinae*	Buckwheat both sides
Encarsia spp.[a]	2.85 ± 0.58a	2.95 ± 0.6a	2.0 ± 0.5a	2.1 ± 0.37a	3.03 ± 0.67a
Eretmocerus spp.[b]	2.25 ± 0.56a	2.6 ± 0.62a	0.9 ± 0.26b	1.4 ± 0.36ab	2.48 ± 0.69a
Orius spp.[c]	1.78 ± 0.39a	1.18 ± 0.33ab	1.3 ± 0.4ab	0.85 ± 0.19b	1.4 ± 0.33ab
Trichogramma spp.[d]	2.7 ± 0.35a	2.48 ± 0.4ab	1.77 ± 0.34bc	2.2 ± 0.32abc	1.73 ± 0.27c

Means in rows followed by the same letter are not significantly different ($P \leq 0.05$).
[a]$F = 1.29$; $df = 4, 165$; $P = 0.2753$.
[b]$F = 3.23$; $df = 4, 165$; $P = 0.0140$.
[c]$F = 2.05$; $df = 4, 165$; $P = 0.0894$.
[d]$F = 2.80$; $df = 4, 165$; $P = 0.0279$.

Table 4. Mean ±*SEM* of natural enemies collected from yellow sticky traps for the intercropping field study in fall 2012 for six treatments: buckwheat alternating (A), buckwheat alternating (A) with *D. catalinae*, buckwheat in the middle (B), buckwheat in the middle (B) with *D. catalinae*, buckwheat on both sides (C), and bare ground.

	Mean number of natural enemies per treatment ±*SEM*					
	Buckwheat alternating	Buckwheat alternating with *D. catalinae*	Buckwheat middle	Buckwheat middle with *D. catalinae*	Buckwheat both sides	Bare ground
Encarsia spp.[a]	3.23 ± 0.63abc	3.13 ± 0.41bc	4.23 ± 0.53a	3.96 ± 0.65ab	2.5 ± 0.42c	2.75 ± 0.45c
Eretmocerus spp.[b]	0.06 ± 0.04b	0.23 ± 0.07ab	0.13 ± 0.05ab	0.17 ± 0.07ab	0.13 ± 0.06ab	0.27 ± 0.09a
Orius spp.[c]	0.23 ± 0.06ab	0.1 ± 0.05ab	0.27 ± 0.14a	0.21 ± 0.07ab	0.17 ± 0.06ab	0.04 ± 0.03b
Trichogramma spp.[d]	4.65 ± 0.48b	4.48 ± 0.42b	3.98 ± 0.54b	4.17 ± 0.47b	4.25 ± 0.43b	8.54 ± 0.89a

Means in rows followed by the same letter are not significantly different ($P \leq 0.05$).

[a] $F = 3.74$; df = 5, 252; $P = 0.0028$.

[b] $F = 1.34$; df = 5, 252; $P = 0.2491$.

[c] $F = 1.53$; df = 5, 252; $P = 0.1809$.

[d] $F = 11.31$; df = 5, 252; $P \leq 0.0001$.

buckwheat B (F = 52.50; df = 1, 252; $P \leq 0.0001$), and buckwheat C (F = 31.17; df = 1, 252; $P \leq 0.0001$) (Table 4).

In 2012, *Encarsia* counts differed by treatment (F = 3.74; df = 5, 252; P = 0.0028) and over time (F = 38.22; df = 5, 252; $P \leq 0.0001$), with no interaction effect (F = 1.05; df = 25, 252; P = 0.4029). There were more *Encarsia* in buckwheat B compared with buckwheat A (F = 4.45; df = 1, 252; P = 0.0359), buckwheat C (F = 12.69; df = 1, 252; P = 0.0004), and the bare ground treatment (F = 8.04; df = 1, 252; P = 0.0049) (Table 4).

In the on-farm demonstration plot (2013), there were no significant differences in the mean number of natural enemy taxa collected from yellow sticky traps among treatments.

The natural enemies collected from pitfall traps included ground beetles (Coleoptera: Carabidae); big-eyed bugs, *Geocoris* spp. (Hemiptera: Lygaeidae); minute pirate bugs, *Orius* spp. (Hemiptera: Anthocoridae); and spiders (Araneae). In 2011 and 2013, there were no significant differences in the mean number of predator taxa collected from pitfall traps among treatments. In 2012, ground beetle counts differed by treatment (F = 3.18; df = 5, 210; $P \leq 0.0001$) and over time (F = 10.29; df = 4, 210; $P \leq 0.0001$), with no interaction effect (F = 1.54; df = 20, 210; P = 0.0695). There were fewer ground beetles in the bare ground treatment compared with all buckwheat treatments, including buckwheat A (F = 14.89; df = 1, 210; P = 0.0002), buckwheat B (F = 8.25; df = 1, 210; P = 0.0045), and buckwheat C (F = 7.38; df = 1, 210; P = 0.0072) (Table 5).

Plant measurements and marketable yields

In 2011, zucchini plant height (cm) differed by treatment (F = 18.64; df = 4, 935; $P \leq 0.0001$), over time (F = 143.61; df = 4, 935; $P \leq 0.0001$), and there was an interaction effect (F = 8.89; df = 16, 935; $P \leq 0.0001$) such that there were treatment differences the first two weeks of sampling. Zucchini plant height was less in buckwheat B compared with

Table 5. Mean ±*SEM* of natural enemies collected from pitfall traps for the intercropping field study in fall 2012 for six treatments: buckwheat alternating (A), buckwheat alternating (A) with *D. catalinae*, buckwheat in the middle (B), buckwheat in the middle (B) with *D. catalinae*, buckwheat on both sides (C), and bare ground.

	Mean number of natural enemies per treatment ±*SEM*					
	Buckwheat alternating	Buckwheat alternating with *D. catalinae*	Buckwheat middle	Buckwheat middle with *D. catalinae*	Buckwheat both sides	Bare ground
Carabidae[a]	0.65 ± 0.11a	0.6 ± 0.14a	0.53 ± 0.12a	0.5 ± 0.15a	0.55 ± 0.14a	0.13 ± 0.06b
Araneae[b]	0.53 ± 0.18ab	0.28 ± 0.11ab	0.3 ± 0.11ab	0.25 ± 0.11b	0.53 ± 0.17ab	0.63 ± 0.22a

Means in rows followed by the same letter are not significantly different ($P \leq 0.05$).
[a]F = 3.18; df = 5, 210; P = 0.0087.
[b]F = 1.06; df = 5, 210; P = 0.3828.

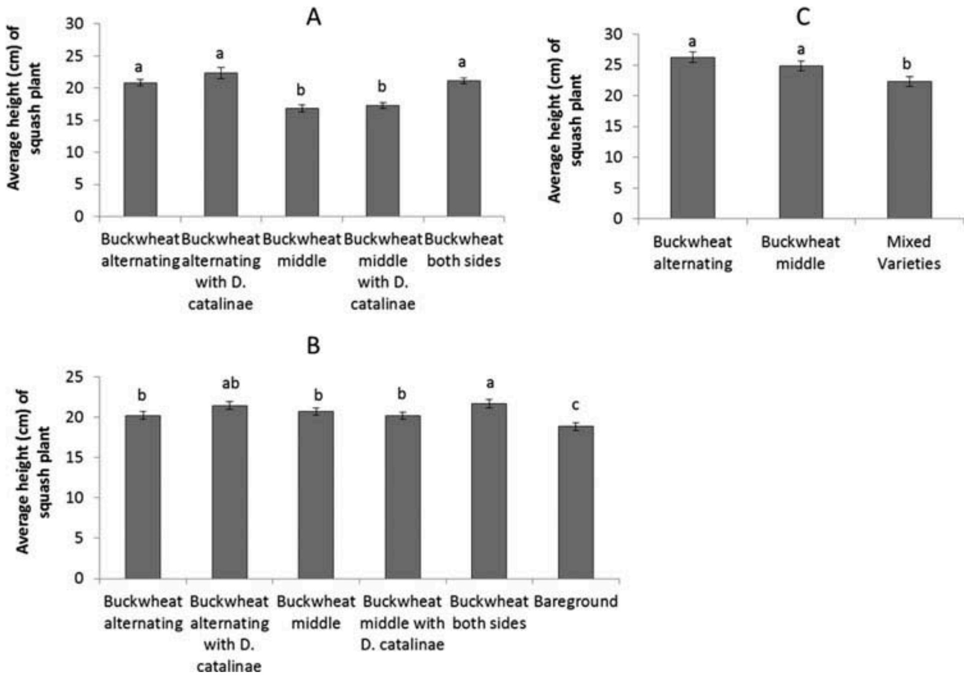

Figure 6. Mean (±*SE*) height (cm) of squash plants sampled over a five-week period for the intercropping field study in (A) 2011, (B) 2012, and (C) 2013. Treatments with the same letter are not significantly different ($P \le 0.05$).

buckwheat A ($F = 60.31$; $df = 1, 935$; $P \le 0.0001$) and buckwheat C ($F = 32.35$; $df = 1, 935$; $P \le 0.0001$) (Figure 6). Zucchini plant width (cm) also differed by treatment ($F = 32.89$; $df = 4, 935$; $P \le 0.0001$) and over time ($F = 104.43$; $df = 4, 935$; $P \le 0.0001$), with no interaction effect ($F = 1.44$; $df = 16, 935$; $P = 0.1138$). Similar to observations recorded on plant height, zucchini plant width was less in buckwheat B compared with buckwheat A ($F = 85.26$; $df = 1, 935$; $P \le 0.0001$) and buckwheat C ($F = 83.53$; $df = 1, 935$; $P \le 0.0001$) (Figure 7).

In 2012, zucchini plant height differed by treatment ($F = 9.19$; $df = 5, 1170$; $P \le 0.0001$), over time ($F = 704.11$; $df = 4, 1170$; $P \le 0.0001$), and there was an interaction effect ($F = 2.14$; $df = 20, 1170$; $P = 0.0025$), such that there were treatment differences the second, third, and fourth weeks of sampling. Zucchini plant height was greater in buckwheat C compared with buckwheat A ($F = 4.19$; $df = 1, 1170$; $P = 0.0408$), buckwheat B ($F = 8.84$; $df = 1, 1170$; $P = 0.0030$), and the bare ground treatment ($F = 35.20$; $df = 1, 1170$; $P \le 0.0001$) (Figure 6). Additionally, plant height was less in the bare ground treatment compared with buckwheat A ($F = 23.07$; $df = 1, 1170$; $P \le 0.0001$) and buckwheat B ($F = 15.04$; $df = 1, 1170$; $P = 0.0001$) (Figure 6). Zucchini plant width differed by treatment ($F = 11.97$; $df = 5, 1170$; $P \le 0.0001$) and over time ($F = 71.47$; $df = 4, 1170$; $P \le 0.0001$), but there was no interaction

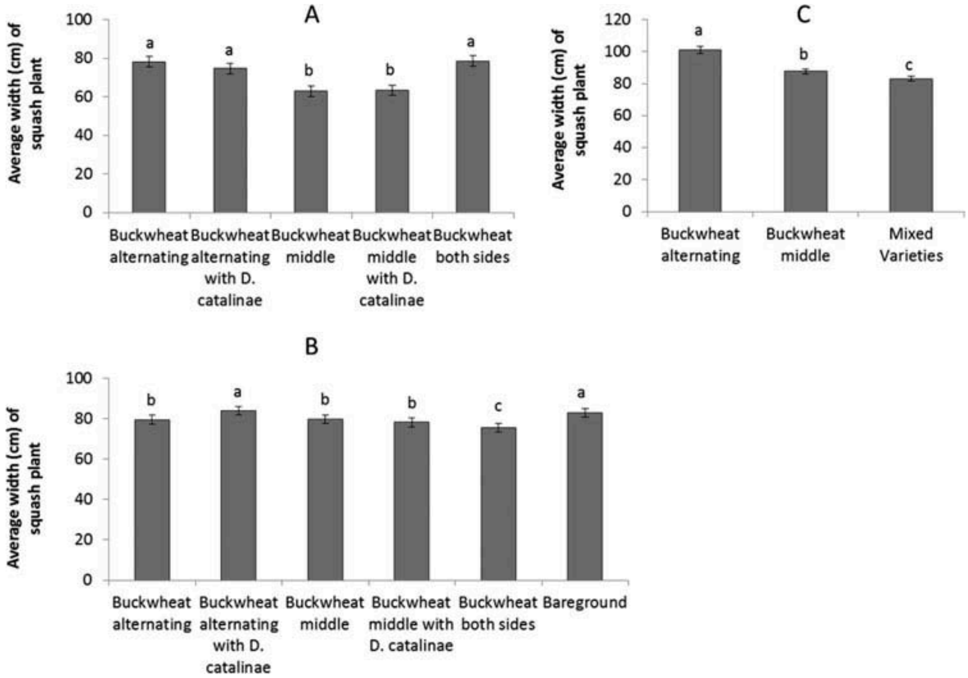

Figure 7. Mean (±*SE*) width (cm) of squash plants sampled over a five-week period for the intercropping field study in (A) 2011, (B) 2012, and (C) 2013. Treatments with the same letter are not significantly different ($P \leq 0.05$).

effect ($F = 0.81$; $df = 20, 1170$; $P = 0.7083$). Zucchini plant width was less in buckwheat C compared with buckwheat A ($F = 34.04$; $df = 1, 1170$; $P \leq 0.0001$), buckwheat B ($F = 9.64$; $df = 1, 1170$; $P = 0.0020$), and the bare ground treatment ($F = 34.39$; $df = 1, 1170$; $P \leq 0.0001$) (Figure 7). Zucchini plant width was also less in buckwheat B compared with buckwheat A ($F = 11.18$; $df = 1, 1170$; $P = 0.0009$) and the bare ground treatment ($F = 13.45$; $df = 1, 1170$; $P = 0.0003$) (Figure 7).

In 2013, squash plant height differed by treatment ($F = 13.13$; $df = 2, 210$; $P \leq 0.0001$), over time ($F = 61.65$; $df = 4, 210$; $P \leq 0.0001$), and there was an interaction effect ($F = 4.07$; $df = 8, 210$; $P = 0.0002$), such that there were treatment differences the fourth and fifth weeks of sampling. Squash plant height was less in the mixed varieties treatment compared with buckwheat A ($F = 25.64$; $df = 1, 210$; $P \leq 0.0001$) and buckwheat B ($F = 10.34$; $df = 1, 210$; $P = 0.0015$) (Figure 6). Squash plant width differed by treatment ($F = 37.01$; $df = 2, 210$; $P \leq 0.0001$), over time ($F = 12.45$; $df = 4, 210$; $P \leq 0.0001$), and there was an interaction effect ($F = 2.25$; $df = 8, 210$; $P = 0.0250$), such that there were treatment differences in all weeks except the first week of sampling. Squash plant width was less in the mixed varieties treatment compared with buckwheat A ($F = 68.58$; $df = 1, 210$; $P \leq 0.0001$) and buckwheat B ($F = 4.50$; $df = 1,$

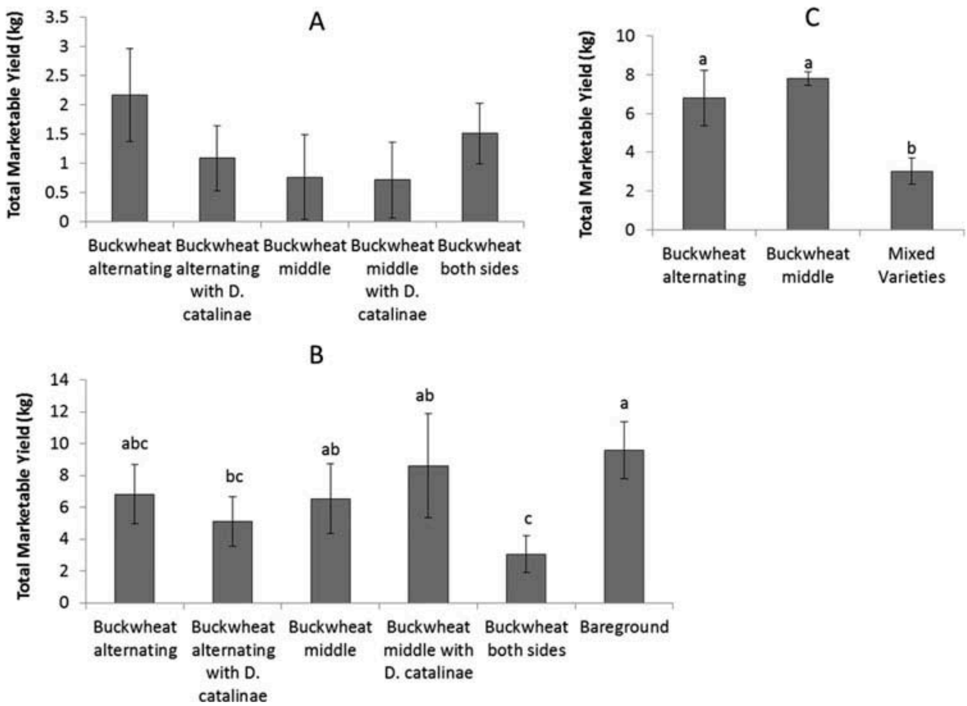

Figure 8. Total marketable squash yield (kg) (±*SE*) harvested for the intercropping field study in (A) 2011, (B) 2012, and (C) 2013. Treatments with the same letter are not significantly different ($P \leq 0.05$).

210; $P = 0.0351$) (Figure 7). Squash plant width was also less in buckwheat B compared with buckwheat A ($F = 37.95$; $df = 1, 210$; $P \leq 0.0001$) (Figure 7).

In 2011, marketable zucchini yields (kg) were not significantly different by treatment ($F = 1.32$; $df = 1, 14$; $P = 0.3092$) (Figure 8). In 2012, marketable zucchini yields (kg) were less in buckwheat C treatment compared with buckwheat B ($F = 5.05$; $df = 1, 18$; $P = 0.0374$) and the bare ground treatment ($F = 7.22$; $df = 1, 18$; $P = 0.0150$) (Figure 8). In 2013, marketable squash yields (kg) were less in the mixed varieties treatment compared with buckwheat A ($F = 8.22$; $df = 1, 6$; $P = 0.0285$) and buckwheat B ($F = 12.70$; $df = 1, 6$; $P = 0.0119$) (Figure 8).

Discussion

Aphids

There were fewer aphids on squash plants in buckwheat C and buckwheat A in the 2012 season. Hooks, Valenzuela, and Defrank (1998) and Nyoike and Liburd (2010) similarly found reduced aphid densities on squash planted with buckwheat when compared with bare ground and white mulch treatments, respectively. It is hypothesized that the buckwheat impairs the aphid's host-finding abilities and deters aphids from landing on the host plant by reducing the host plant's apparency to the pest (Root 1973). There were also fewer aphids on the mixed variety treatment, which represented the grower's standard control, compared with buckwheat treatments. Although planting multiple squash cultivars has not been studied, Ninkovic, Olsson, and Pettersson (2002) found that aphid acceptance in barley cultivars was significantly reduced when sown together with other cultivars compared with pure stands, such that the volatiles released from a neighboring cultivar plant could change aphid host plant acceptance in another cultivar. They suggested that plant/plant communication (i.e., volatiles) could be an important mechanism affecting aphid acceptance of a host. Overall, aphid densities were reduced in treatments where buckwheat was present and where multiple varieties of squash were planted. Therefore, intercropping buckwheat with squash and incorporating multiple squash varieties could be effective strategies for suppressing aphid populations.

Whiteflies

In 2011, immature whitefly densities were reduced on squash plants in buckwheat A compared with buckwheat C. However, in 2012, whitefly pressure was slightly lower and there was a significant reduction in immature whitefly densities on squash plants in the bare ground treatment compared with buckwheat treatments. This finding may be related to the time of establishment for the buckwheat crop 10 d before planting squash in 2011 versus 3 d in 2012, such that more established buckwheat plants could act to deter whitefly colonization on squash plants. A second hypothesis is that immature whitefly populations could be more apparent to beneficial organisms in a less diversified cropping system (Pimentel 1961; Root 1973), and, therefore, natural enemies present in the bare ground treatment may be more effective at detecting the presence of whiteflies and consequently reducing their populations. In 2013, fewer immature whiteflies were recorded on the mixed variety treatment compared with buckwheat treatments, which is similar to the findings of reduced aphid densities on mixed squash variety plantings. Planting multiple squash varieties could have a deterrent effect on whitefly populations, but this hypothesis needs further testing. Furthermore, one or more of the squash plant cultivars that were selected by the grower in the mixed variety treatment could be expressing genotypes conferring resistance to aphid and whitefly pests. Coffey et al. (2015) identified several melon genotypes that exhibited high levels of resistance to *B. tabaci* in laboratory and greenhouse experiments, and they suggested that these genotypes could be useful in breeding projects aiming to improve whitefly resistance in watermelon cultivars.

Diseases

In 2011, there was a high incidence of *Cucurbit leaf crumple virus*, where each plot had over 25% of plants showing virus symptoms, and virus incidence was not significantly different between treatments. The field plots were bordered by several other cucurbit crops that were planted earlier in the 2011 season, including pumpkins, watermelon, and melon. It is hypothesized that high virus incidence was a result of viruliferous whitefly adults immigrating into the field from the bordering cucurbit fields. SSL incidence was greater in buckwheat C. Consequently, a greater number of immature whiteflies were also recorded on squash plants in buckwheat C and were presumed to be responsible for inducing silverleaf symptoms (Schuster, Kring, and Price 1991). In 2012, virus incidence was reduced in all buckwheat treatments when compared with the bare ground treatment. However, SSL incidence was reduced in the bare ground treatment, presumably due to the lower numbers of immature whiteflies. In the on-farm demonstration (2013), virus symptoms and SSL were reduced in the mixed varieties treatment, which correlates with lower aphid and whitefly densities.

Our results suggest that buckwheat as a living mulch can reduce the incidence of insect-transmitted viruses in zucchini squash when compared with bare ground treatments, as supported by earlier work from Hooks, Valenzuela, and Defrank (1998) and Nyoike, Liburd, and Webb (2008). However, buckwheat was not as effective at reducing SSL incidence, and a greater incidence of SSL may be related to a higher population of immature whiteflies (Yokomi, Hoelmer, and Osborne 1990).

Natural enemies

In 2011 and 2012, lacewing (Chrysopidae) counts were lower on squash plants in both the bare ground treatment and buckwheat C compared with buckwheat A and buckwheat B. The lower population of lacewings in the bare ground treatment may suggest that buckwheat attracted lacewings; however, in buckwheat C where buckwheat was planted continuously on both sides of the squash, the buckwheat may have acted as a barrier, deterring oviposition by lacewings on squash plants. In 2011, lacewing counts were lower in treatments where *D. catalinae* was released, and it is hypothesized that intraguild predation may have been a factor. Since both *D. catalinae* and Chrysopidae species are competing for limited resources (hemipteran eggs, nymphs, and adults) this can result in a lower population of Chrysopids in the field due to egg or nymph consumption if *D. catalinae* is the dominant predator. Heinz et al. (1999) reported negative predator-predator interactions in field evaluations in cotton between *D. catalinae* and other predators including *Chrysoperla*, *Geocoris*, and *Orius* spp. In addition, the findings from 2012 suggest that both *Geocoris* spp. and *Orius* spp. counts were higher on squash plants in buckwheat treatments, which supports the hypothesis that intercropping can enhance natural enemy populations (Bugg 1991). Ground beetle (Carabidae) counts were also higher in buckwheat treatments in 2012 compared with the bare ground treatment. Prasifka et al. (2006) also reported that living mulches integrated into a corn-soybean-forage crop rotation positively impacted ground beetle densities and suggested that the additional groundcover provided by living mulches can increase ground beetle populations.

Both *Encarsia* spp. and *Eretmocerus* spp. were more abundant in buckwheat A and buckwheat C compared with buckwheat B in 2011. Alternatively, in 2012, *Encarsia* spp. were more abundant in buckwheat B compared with the other treatments, where immature whitefly densities were high, but not different from other buckwheat treatments. In contrast, *Trichogramma* spp. counts were greater in the bare ground treatment compared with buckwheat treatments. In treatments where buckwheat was absent, the pest or the host plant of the pest could have been more apparent to the parasitoid (Pimentel 1961; Root 1973) and facilitated greater host finding abilities, particularly of melonworm, *Diaphania hyalinata* L. and other lepidopteran eggs that *Trichogramma* spp. are known to parasitize (Hassan 1994). Generally, natural enemy abundance varied between treatments, and we hypothesize this was likely due to differences between natural enemy species in host-finding behavior and their dependence on alternative resources. The different buckwheat arrangements may have also affected host-finding behavior. For instance, many *Encarsia* spp. and *Eretmocerus* spp. use chemical cues, such as volatile semiochemicals from their host or whitefly-induced plant volatiles, during host searching (Birkett et al. 2003; Heinz and Parrella 1998). The search behavior exhibited by *Encarsia* and *Eretmocerus* could allow for greater efficiency of finding a whitefly host on squash surrounded by buckwheat compared to the host search behavior of *Trichogramma* spp., which relies more heavily on visual and chemical cues from the host (Knutson 2005). Therefore, host species may be easier to locate on squash not surrounded by other plants. In 2013, we did not observe significant differences in natural enemy counts in on-farm demonstration plots, which may have been a result of smaller plot sizes and increased movement between plots.

There were no differences among treatments with similar intercropping tactics when considering the effect of *D. catalinae* on pest populations, disease incidence, and zucchini yield. This may suggest that there was movement of *D. catalinae* between plots into areas where it is not released. Heinz et al. (1999) reported dispersal by *D. catalinae* was less than 1 m/day and suggested that movement out of experimental plots should be minimal.

However, Hoelmer and Pickett (2003) reported a high degree of dispersal by *D. catalinae* in the field, which is consistent with the observations for this study. Therefore, it was difficult to determine the effectiveness of *D. catalinae* on whitefly populations in the field. Future research should consider movement and colonization of *D. catalinae* and alternative methods to evaluating the effect of *D. catalinae* on whitefly populations in the field, such as increasing the distance between treatments and the use of exclusion cages around treatment plots.

Plant measurements and marketable yields

In all three years, the squash plants in buckwheat B were significantly smaller than the plants in buckwheat A. We hypothesize that competition between squash and buckwheat was greater in buckwheat B than in buckwheat A, which may account for the smaller plant size. Squash plants in buckwheat C were taller than in buckwheat B and the bare ground treatment. The presence of buckwheat on both sides of the squash plants, as in buckwheat C, forced plants to grow taller instead of wider. It was also observed that squash plant height in the bare ground treatment was reduced compared with plants in buckwheat treatments; however plant width was greater in the bare ground treatment compared with buckwheat B and buckwheat C. It is hypothesized that the absence of buckwheat allowed squash plants to grow wider instead of being forced to grow taller. Squash plant size in buckwheat A was not significantly reduced compared with plants in the bare ground treatment, which suggests that competition between buckwheat and the zucchini crop was minimized in buckwheat A. Overall, squash plants in the mixed varieties treatment in the on-farm demonstration were smaller than the other treatments. This observation suggests that the varieties selected for the mixed varieties treatment were not as vigorous as the variety used in the buckwheat treatments.

In 2011, marketable yields were not different between treatments, and it is hypothesized that high virus incidence contributed to low yields across treatments. A second hypothesis is that by planting the buckwheat 10 d earlier than the squash, early-season competition between buckwheat and squash may have been high and could have contributed to reduced yields. In 2012, marketable yields were reduced in buckwheat C compared with the other treatments. Nyoike and Liburd (2010) similarly reported high competition between buckwheat and zucchini squash for this arrangement, which resulted in smaller plant size and reduced yields. However, marketable yields were not different between the bare ground treatment and buckwheat A and B. This finding suggests that manipulating the buckwheat spacing within the squash crop can minimize the competition between the main crop and the living mulch and potentially improve yields.

In 2013, a reduction in marketable yields in the mixed varieties treatment was noted. The varieties the growers used for this treatment were not as high yielding as the other varieties utilized in the buckwheat treatments. However, observations of a significant reduction in pest densities and virus incidence in the mixed varieties treatment suggest that this could be an effective strategy if higher yielding varieties are incorporated.

In conclusion, this study should be useful in providing information on how intercropping tactics can be utilized to maximize yields in squash and other cropping systems. An important finding from this intercropping research was that manipulations in space and time can serve to reduce competition between the living mulch and the main crop and ultimately increase marketable yields. While marketable yields were not significantly different between buckwheat A and B, squash plants were larger in buckwheat A compared with buckwheat B. Based on conversations with producers and farm managers, buckwheat A was preferred over buckwheat B in terms of planting ease and reduction in labor costs. Therefore, buckwheat A is recommended for intercropping buckwheat and squash for the purpose of reducing competition between buckwheat and squash plants and providing a more cost-effective option when integrating buckwheat into an organic squash production system.

While reduced pest and disease incidence was observed in buckwheat treatments during the three-year study, observations were not consistently different compared to the bare ground treatment. Hooks and Wright (2008) found adult and immature whitefly numbers to be similar among bare ground and mulch treatments in zucchini squash. They suggested that in areas where whitefly densities are high, as is the case during the fall growing season, buckwheat may not be a feasible barrier plant. However, when used in conjunction with other pest management tactics, enhanced pest and disease suppression could be achieved. With the added benefits of increased natural enemy densities that were observed in buckwheat and the addition of insecticides approved for organic production, a reduction in pest and disease pressure and an increase in yields could be achieved. Therefore, future research should evaluate the effectiveness of organic insecticides on aphid and whitefly populations and how this pest management strategy can be incorporated into an IPM program utilizing living mulches in organic squash production systems.

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