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## **Effect of Cover Crops on Aphids, Whiteflies, and Their Associated Natural Enemies in Organic Squash**

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*Field experiments conducted in fall 2006 and 2007 evaluated the effects of monoculture and diculture cover crops on aphids, whiteflies and beneficials in organic squash. Insect populations were assessed using in situ and leaf disc counts, blue pan traps and unbaited yellow sticky traps. High levels of parasitoids and coccinellids were found in sorghum sudangrass (SSG) plots. The grass monoculture pearl millet had lower aphid populations than other treatments in 2006. The diculture sunnhemp/pearl millet treatment and the grass cover crop SSG had low whitefly populations in 2006. Therefore, the potential use of cover crops in organic agriculture is discussed.*

**KEYWORDS** *cover crops, organic system, aphids, whiteflies, legumes, grasses*

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## INTRODUCTION

In the United States, Florida is the leading producer of summer squash (*Cucurbita pepo* L.) with an annual value of US \$57 million in 2010 (U.S. Department of Agriculture, National Agricultural Statistics Service 2011). Many cucurbits including summer squash are severely affected by a wide range of key agricultural pests including several species of aphids, and whiteflies, *Bemisia tabaci* B-biotype (Gennadius). Hemipteran pests including aphids and whiteflies are some of the most economically destructive pests worldwide causing great damage to horticultural crops, ornamentals, row crops, greenhouse plants, and vegetables (McAuslane et al. 1996; Goolsby et al. 1998; Simmons et al. 2000; Nebreda et al. 2005). They suck sap juices from plants, excrete honeydew, which promotes sooty mold growth, and transmit numerous viral diseases, all of which reduce plant vigor and marketable yields (Liburd and Frank 2007).

Aphid species affecting squash include the green peach aphid, *Myzus persicae* (Sulzer), cotton aphid, *Aphis gossypii*, Glover, and cowpea aphid, *Aphis craccivora* Koch (Mossler and Nesheim 2001). They have high growth and developmental rates (Dixon 1977) that can result in high populations over a relatively short period of time. Signs of infestation include leaf distortions, reduced fruit set, yellowing, mosaic and leaf blistering (Frank and Liburd 2005; Vásquez et al. 2006). Aphids also vector numerous viruses, which affect cucurbits including *Papaya ringspot virus* type W (PRSV-W), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV), and the *Cucumber mosaic virus* (CMV; Baker et al. 2008).

Whiteflies are generally multivoltine, having 4–6 generations per year in Florida. However, they can continue to reproduce as long as temperatures are favorable (Byrne and Bellows 1991). The silverleaf whitefly, *Bemisia tabaci* B-biotype (Gennadius) is the key whitefly species that feeds on field-grown squash causing squash silverleaf (SSL) disorder. This disorder causes dramatic reductions in marketable yield (Hooks et al. 1998; Nyoike and Liburd 2010). Signs of infestation include premature dehiscence, defoliation, leaf chlorosis, leaf withering, and ultimately plant death (Byrne and Bellows 1991). The silverleaf whitefly also transmits several viral diseases in squash including the *Squash Vein Yellowing Virus* (SqVYV), *Cucurbit Yellow Stunting Disorder Virus* (CYSDV), and the *Cucurbit Leaf Crumple Virus*, a recently discovered whitefly virus in Florida (Nyoike et al. 2008).

Generally, aphids and whiteflies in squash are controlled in conventional systems using broad-spectrum insecticides from several classes (Palumbo et al. 2001; Webb 2006; Dewar 2007). Some of these insecticides threaten non-target organisms including natural enemies, and have negative effects on the environment. In addition, synthetic insecticides may enhance the spread of non-persistent viruses (Stapleton et al. 2002). Neonicotinoids are one of the newer class of insecticides that are effective against aphids

and whiteflies. However some aphid and whitefly species have demonstrated potential for resistance to these insecticides (Foster et al. 2002; Nauen et al. 2002).

Organic farms use cultural and biological control techniques to manage key insect pests. Cover crops are an integral component of organic farming and sustainable agriculture (Liburd et al. 2008) and can be used in several ways for insect management practices (Bugg 1991). Cover crops reduce colonization, dispersal, and reproduction of pests in vegetable crops by acting as a sink (Bugg 1992). In addition when intercropped with a cash crop, cover crops enhance the natural enemy populations (Frank and Liburd 2005; Liburd et al. 2008; Nyoike and Liburd 2010). To date, there has been no studies that investigate the effects of incorporating cover crops into the soil for potential pest suppression in a subsequent cash crop.

The primary goal of this research was to study the population dynamics of aphids, whiteflies, and beneficial insects in organic squash treated with monoculture (one cover crop species) and diculture (combination of two cover crop species) cover crops that were incorporated into the soil. It is hypothesized that natural enemies from cover crops will immigrate to nearby refugia of secondary hosts until the cash crop is established. They will then leave the refugia in preference for pests on the host plant or cash crop.

## MATERIALS AND METHODS

The experiment was conducted at the University of Florida Plant Science Research and Education Unit in Citra in 2006 and repeated in 2007. The experimental design was a randomized complete block with four replicates of seven cover crop treatments. Monoculture treatments included two legumes, sunn hemp [SH] (*Crotalaria juncea* L.) and velvet bean [VB] (*Mucuna pruriens* (L.) cv. GA Bush), and two grasses, sorghum sudangrass [SSG] (*S. bicolor* × *S. sudanense* cv. Brown-Midrib) and pearl millet [PM] (*Pennisetum glaucum* L. cv. Tifleaf3). Diculture treatments included two legume/grass mixtures, sunn hemp/pearl millet [SH/PM] and sorghum sudangrass/velvet bean [SSG/VB], and a weedy fallow (control). Each treatment plot measured 12 × 12 m, and was separated by mowed alleys that were 12 m wide, which was intended to reduce the movement of pest and beneficial arthropods between plots.

Both legumes, SH and VB, were broadcast at a rate of 40 lbs/ acre (218 kg/ha) and 100 lbs/ acre (545 kg/ha), respectively, while PM and SSG were each planted in rows approximately 18 cm apart at the rate of 25lbs/acre (153 kg/ha) and 40 lbs/acre (218 kg/ha), respectively. The SH/PM mixture was planted using a Sukup 2100 Planter at half of the recommended seed rate of SH, 20 lbs/acre (109 kg/ha) plus 2/3 the recommended seed rate of PM, 17 lbs/acre (93 kg /ha).

## Plot Preparation

Prior to the beginning of the experiment in 2006, the experimental area was treated with mushroom compost (Quincy Farms, 190 Mannie Gunn Rd., Quincy, FL, USA) at 10 tons/acre (2245 kg/ha) to enhance soil nutrient content. Southern cowpea, *Vigna unguiculata* L. cv. White Acre, (Alachua Seed and Lumber, Alachua, FL, USA) cover crop was incorporated into the soil using a John Deere 6615 tractor (Marion Tractor, Inc., Ocala, FL, USA) at 50 lbs/acre (272.5 kg/ha) at a depth of 5 cm prior to establishing the cover crop treatments to improve soil and nutrient fertility.

In 2007, three weeks prior to cover crop planting, 90% elemental sulfur (Tiger 90; Tiger-Sul Products, Atmore, AL, USA) was applied to the entire field at 8 lbs/plot (3.6 kg/plot) with a drop spreader (Newton Crouch Inc., Griffin GA, USA) to reduce the alkalinity of the soil. One application of Sul-Po-Mag at 8.2 lbs/plot (3.71 kg/plot) was also applied to all treatment plots 24 h before cover crop planting.

## Cucurbit Planting

In 2006, the cover crop treatments were flail-mowed after eight weeks and incorporated into the soil 25.4 cm by disking (New Holland Flail Mower, Purdy Tractor and Equipment Inc., Hillsdale, MI, USA). Yellow squash (Cougar F1 cultivar, Harris seeds) was direct seeded into each treatment plot on October 19, 2006, three weeks after cover crop incorporation. Each plot measured 12 × 12 m with 12 rows of squash. However, due to the poor germination of the squash plants, areas with no emergence had to be reseeded. Reseeding was done on October 30, 2006. Arthropod sampling began 4 weeks after reseeding. In 2007, squash plants were direct seeded on October 10, 2007; 7 d after cover crops were incorporated. Insect sampling in squash began 4 weeks after planting.

## Sampling

Aphids, whiteflies, and beneficials in squash were sampled using in situ counts, leaf disc counts, unbaited yellow sticky traps, blue pan traps, and pitfall traps. Sampling dates were similar in 2006 and 2007. In 2006, sampling started on November 17, continued weekly for three weeks, and ended on December 11. In 2007, sampling started on November 8, continued weekly for approximately four weeks, and ended on December 5.

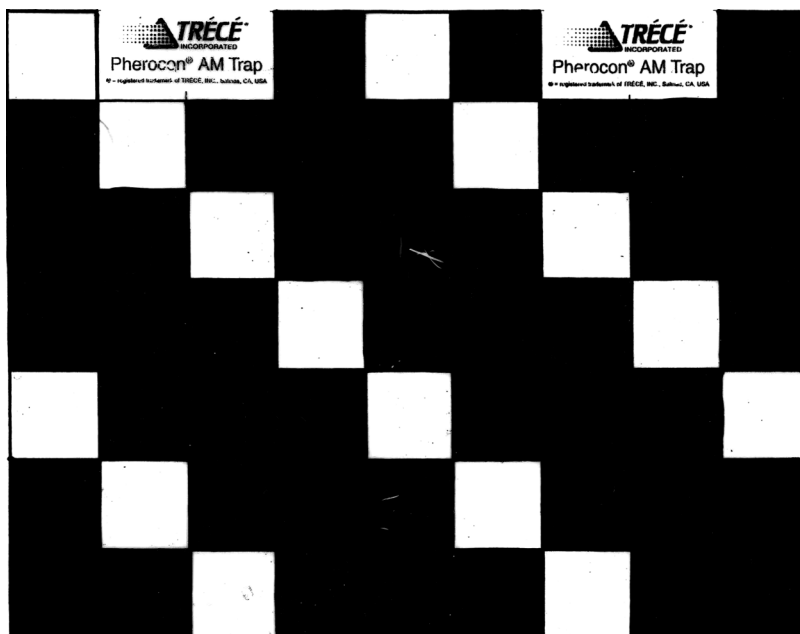
### APHIDS AND WHITEFLIES

Each week visual (in situ) counts were conducted in the field to record the number of aphids (alate and apterous), whiteflies (adults), as well as other

insects. Leaves of eight squash plants were visually assessed for insects. Approximately 1 min was spent randomly assessing the leaves of each squash plant from the two inner rows.

Circular leaf discs were also used to estimate the number of whitefly nymphs and eggs on squash plants (Frank and Liburd 2005). Every two weeks, eight plants were randomly chosen from the two inner rows and one leaf was randomly picked from each of the eight plants and taken back to the University of Florida Small Fruit and Vegetable IPM Laboratory. Two circular (2.5 cm diameter) sections were cut from each leaf using a cork borer. Leaf discs were then examined under a 40 $\times$  dissecting microscope for whitefly eggs and immatures (Frank and Liburd 2005).

Aphid and whitefly adults were also assessed with yellow sticky traps by using a subsampling technique as outlined by Liburd et al. (2009). This subsampling technique eliminates counting errors and reduces the time spent per trap. Briefly, each yellow sticky trap was overlaid with a gridded transparent paper divided into 63, 1-inch squares. Forty-eight of these squares were colored in, while 15 were left transparent (Figure 1). The area under these 15 transparent 1-inch squares was examined under a 40 $\times$  dissecting microscope and the number of aphids and whiteflies were recorded.



**FIGURE 1** Gridded transparency used to count aphids, whiteflies, and parasitoids on unbaited yellow sticky traps.

## NATURAL ENEMIES

Natural enemies were monitored using sticky traps and blue pan traps. In 2006 and 2007, parasitoids and coccinellids in each squash plot were monitored using one unbaited yellow sticky trap (Great Lakes IPM, Vestaburg, MI, USA). Traps were left in the field for 48 h then brought back to the University of Florida, Small Fruit and Vegetable IPM Laboratory to be processed. The number of parasitoids on each sticky trap were counted and recorded. Parasitoid numbers were low therefore; the parasitoids in all 63 squares on the sticky traps were counted and recorded for each unbaited yellow trap.

Syrphid flies were monitored using blue pan traps (Packer Ware Bowls, Gainesville, FL, USA). Blue traps were supported by tomato wire cages (45 × 15 × 20 cm), filled with water and detergent [5% detergent solution] (Colgate Palmolive Co., New York, NY, USA) to break the surface tension (Webb et al. 1994). One blue pan trap was placed diagonally across from an unbaited yellow sticky trap on the opposite end on the plot. Traps were left in the field for one week. At the end of the week, the contents from each trap were emptied into a small plastic container, labeled accordingly and taken back to the University of Florida, Small Fruit and Vegetable IPM Laboratory to be analyzed. Syrphid flies from blue traps were counted by species under a 40× dissecting microscope and the contents placed in 15 × 45 mm vials (Fisherbrand, Fisher Scientific, Pittsburgh, PA, USA) with 70% ethanol for storage.

Both yellow sticky traps and blue pan traps were placed in the inner two rows of the squash plots during sampling to eliminate edge effects.

## Sentinel Squash Plants and Peripheral Sampling

Based on the results from 2006, and to gain a better understanding on the movement of pests and natural enemies after cover crops were incorporated into the soil, sentinel plants were established in 2007 to track the movement of pest and beneficial insects. Sentinel squash plants were grown in 6-inch pots, filled with potting mixture (Jungle Growth, Stathom, GA, USA) at the Small Fruit and Vegetable IPM Greenhouse at the University of Florida using standard squash production practices (Olsen and Santos 2010). Pots were given drip irrigation for three weeks before being transported to the field. Pots were transported to the field on the day cover crops were incorporated into the soil.

Two potted sentinel squash plants were placed on the edge of each plot 2 h following cover crop incorporation into the soil. Sentinel plants were placed in the plots on October 3, 2007, and removed from the field on October 15, 2007. Sentinel squash plants were approximately 30 cm in

height when placed in the field. Plants were placed diagonally across from each other (approximately 15 m apart) within in each treatment plot. For each sentinel squash plant, aphids, whiteflies, and natural enemies were assessed via in situ counts once per week for two weeks. Similar to the squash sampling that was discussed earlier, plants were observed for one minute and the number of insects (pests and beneficials were recorded). On the third week, the sentinel plants were removed to allow for direct seeding of squash into the treatment plots.

In addition to sentinel squash plants, 20 unbaited yellow sticky traps were placed on the periphery of the field to track natural enemy populations after incorporation of cover crop treatments (prior to planting squash). Traps were placed along the periphery of the field on the October 3, 6, and 9, 2007. Traps were approximately 50 m apart. Traps remained in their peripheral location for 48 h. They were then removed from the field, covered with plastic wrap and brought back to the Small Fruit and Vegetable IPM Laboratory at the University of Florida for processing.

The vegetation bordering the field and adjacent to sentinel plants was divided into three types: 1) fruit trees (orange), 2) wooded area of various trees and shrubbery, and 3) grass/weeds.

### Squash Silver Leaf Disorder Rating

Unlike 2006, in 2007, we noticed squash silver leaf (SSL) disorder in the field and recorded the severity of the disorder based on the following scale 0 = no silvering (0% silvering), 3 = moderate silvering (50% silvering), 5 = complete silvering (100% silvering; Frank and Liburd 2005). Four plants from the middle of the inner two rows of each plot were sampled and rated based on the above scale. Sampling was conducted two weeks after planting and at the end of the field-season (eight weeks after planting).

### Statistical Analysis

Data from all sampling techniques along with the SSL disorder rating were square root transformed and analyzed using analysis of variance (ANOVA) PROC GLM (SAS Institute 2003). Treatment means were separated using the least significant difference (LSD). Results were considered significant if  $P \leq 0.05$ .

Key pest (aphid and whitefly) data from peripheral yellow sticky traps were also analyzed using analysis of variance (ANOVA) PROC GLM (SAS Institute 2003) because there was no interaction between habitat and time (sampling weeks). However, parasitoid data from peripheral traps were analyzed using repeated measures analysis (PROC MIXED, SAS Institute 2003) to show interaction effect between the different habitats and time (sampling



weeks). Regression analysis (PROC REG, SAS Institute 2003) was also carried out to assess the relationship between the number of whitefly immatures and the presence of SSL disorder.

## RESULTS

### Effect of Cover Crops on Aphid Population on Peripheral Areas and Organic Squash

#### APHIDS ON SENTINEL SQUASH PLANTS AND IN PERIPHERAL AREAS

The principal aphid species recorded in peripheral areas and on organic squash was the melon aphid, *Aphis gossypii* Glover. Other rare occurrences include the green peach aphid, *M. persicae* (Sulzer).

The peripheral vegetation was divided into three distinct areas 1) a fruit tree area to the west of the field, 2) a wooded area to the north of the field, and 3) a grassy/weedy area to the east and south of the field. Following the incorporation of cover crops we found that there were significantly more aphids in the wooded habitat compared with the other habitats ( $F = 9.08$ ;  $df = 2, 4$ ;  $P = 0.0087$ ). There was no difference in aphid numbers between the fruit trees and the grassy areas (Table 1).

#### ORGANIC SQUASH

**2006:** In situ counts of aphids revealed that squash planted in several plots including grass and legume monocultures (PM, SSG, and VB) and the diculture legume/grass mixture (SSG/VB) had significantly lower aphid populations compared with the weedy fallow ( $F = 6.21$ ;  $df = 3, 6$ ;  $P = 0.0011$ ) (Table 2). Squash planted in plots previously treated with legume-grass combination SH/PM, and SH had high aphid numbers that were not significantly different from the weedy fallow control (Table 2).

**TABLE 1** Mean number of aphid and whitefly adults from peripheral habitats following incorporation of cover crops in an organic field (2007)

Habitats	Mean $\pm$ SEM key pests	
	Aphids <sup>a</sup>	Whiteflies <sup>b</sup>
Fruit tree (oranges)	10.80 $\pm$ 0.66b	7.00 $\pm$ 0.94b
Grass	9.40 $\pm$ 1.91b	3.00 $\pm$ 0.71b
Woods	19.20 $\pm$ 2.65a	32.40 $\pm$ 4.05a

The letters a and b refer to significantly different means. Means followed by the same letters are not significantly different  $P = 0.05$  (LSD).

<sup>a</sup> $F = 9.08$ ;  $df = 2, 4$ ;  $P = 0.0087$ .

<sup>b</sup> $F = 47.20$ ;  $df = 2, 4$ ;  $P < 0.0001$ .

**TABLE 2** Mean  $\pm$  SEM number of aphids found on organic squash leaves in various cover crop treatments

Treatment	2006 <sup>a</sup>	2007 <sup>b</sup>
SH	157.00 $\pm$ 7.71ab	161.75 $\pm$ 6.82d
PM	129.75 $\pm$ 6.90bc	216.00 $\pm$ 10.31c
SH + PM	176.25 $\pm$ 1.79a	346.25 $\pm$ 14.67a
SSG	130.50 $\pm$ 14.5bc	250.75 $\pm$ 10.81b
VB	119.50 $\pm$ 4.97c	339.00 $\pm$ 13.34a
SSG + VB	122.00 $\pm$ 10.87c	326.00 $\pm$ 18.04a
Weedy fallow (control)	168.00 $\pm$ 8.68a	224.00 $\pm$ 3.48bc

Aphid data were square root transformed before analysis but means shown reflect untransformed data. The letters a and b refer to significantly different means. Means followed by the same letters are not significantly different  $P = 0.05$  (LSD).

<sup>a</sup> $F = 6.21$ ;  $df = 3, 6$ ;  $P = 0.0011$ .

<sup>b</sup> $F = 40.5$ ;  $df = 3, 6$ ;  $P < 0.0001$ .

**2007:** In situ counts revealed that squash planted in plots previously treated with the monoculture SH cover crops had the lowest aphid population compared with all other treatments including the weedy fallow control ( $F = 40.5$ ;  $df = 3, 6$ ;  $P < 0.0001$ ) (Table 2). Also, squash planted in plots previously PM monoculture had significantly fewer aphids than the grass monoculture of SSG, ( $F = 40.5$ ;  $df = 3, 6$ ;  $P < 0.0001$ ). Neither PM nor SSG were significantly different from the control. Overall, squash plants in plots with the legume monoculture VB and legume-grass dicultures SH/PM and SSG/VB had significantly higher number of aphids compared with the weedy fallow control (Table 2).

## Effects of Cover Crops on Whitefly Populations in Organic Squash

### WHITEFLIES ON SENTINEL SQUASH PLANTS AND IN PERIPHERAL AREAS

Whitefly adults in the wooded areas were significantly higher than those recorded in the areas with fruit trees or grasses ( $F = 47.20$ ;  $df = 2, 4$ ;  $P < 0.0001$ ). There was no difference in whitefly numbers between fruit trees and the grassy area (Table 1).

### ORGANIC SQUASH

**2006:** In situ counts in organic squash revealed that plots previously treated with grass monocultures PM and SSG, and the legume-grass diculture SH/PM had significantly fewer adult whiteflies compared with the weedy fallow control ( $F = 7.61$ ;  $df = 3, 6$ ;  $P = 0.0004$ ). Squash planted in plots where VB was incorporated into the soil had significantly more adult whiteflies compared with the control. None of the other treatments were significantly different to the control (Table 3).

**TABLE 3** Mean  $\pm$  SEM number of adult whiteflies found on organic squash leaves in various cover crop treatments

Treatment	2006 <sup>a</sup>	2007 <sup>b</sup>
SH	241.50 $\pm$ 30.44bc	65.25 $\pm$ 7.18cd
PM	169.50 $\pm$ 21.40d	95.57 $\pm$ 9.46ab
SH + PM	196.00 $\pm$ 11.00cd	70.25 $\pm$ 6.51cd
SSG	182.25 $\pm$ 9.63cd	80.25 $\pm$ 8.46bcd
VB	344.00 $\pm$ 33.49a	78.00 $\pm$ 6.25bcd
SSG + VB	223.00 $\pm$ 13.22bcd	103.50 $\pm$ 14.31a
Weedy fallow (control)	260.00 $\pm$ 24.77b	63.00 $\pm$ 5.11d

Whitefly data were square root transformed before analysis but means shown reflect untransformed data. The letters a and b refer to significantly different means. Means followed by the same letters are not significantly different  $P = 0.05$  (LSD).

<sup>a</sup> $F = 7.61$ ;  $df = 3, 6$ ;  $P = 0.0004$ .

<sup>b</sup> $F = 3.10$ ;  $df = 3, 6$ ;  $P = 0.0289$ .

**TABLE 4** Mean  $\pm$  SEM number of whitefly eggs and immatures recorded from squash leaf disc counts

Treatment	Eggs		Immatures	
	2006 <sup>a</sup>	2007 <sup>b</sup>	2006 <sup>c</sup>	2007 <sup>d</sup>
SH	142.50 $\pm$ 3.30	1.25 $\pm$ 0.63c	16.00 $\pm$ 1.29ab	1.75 $\pm$ 1.18b
PM	153.00 $\pm$ 12.04	16.5 $\pm$ 3.32b	12.00 $\pm$ 4.41bc	16.75 $\pm$ 1.93a
SH + PM	121.50 $\pm$ 19.56	2.25 $\pm$ 1.31c	4.75 $\pm$ 1.65c	14.25 $\pm$ 2.86a
SSG	170.25 $\pm$ 8.16	1.00 $\pm$ 0.71c	9.50 $\pm$ 3.30bc	1.75 $\pm$ 1.18b
VB	174.25 $\pm$ 19.73	25.25 $\pm$ 5.57a	14.00 $\pm$ 5.01abc	2.75 $\pm$ 1.88b
SSG + VB	94.75 $\pm$ 15.78	2.75 $\pm$ 1.25c	12.75 $\pm$ 3.11abc	3.00 $\pm$ 2.12b
Weedy fallow (control)	178.00 $\pm$ 41.12	16.00 $\pm$ 5.88b	25.00 $\pm$ 4.02a	5.75 $\pm$ 1.03b

The letters a and b refer to significantly different means. Means followed by the same letters are not significantly different  $P = 0.05$  (LSD). Whitefly immature data were square root transformed before analysis but means shown reflect untransformed data.

<sup>a</sup> $F = 2.41$ ;  $df = 3, 6$ ;  $P = 0.0690$ .

<sup>b</sup> $F = 10.06$ ;  $df = 3, 6$ ;  $P < 0.0001$ .

<sup>c</sup> $F = 3.04$ ;  $df = 3, 6$ ;  $P = 0.0312$ .

<sup>d</sup> $F = 14.44$ ;  $df = 3, 6$ ;  $P < 0.0001$ .

With regard to leaf disc counts there was no significant difference among whitefly eggs on squash plants in 2006 (Table 4). For whitefly immatures in 2006, squash plants from monoculture grass cover crops SSG and PM as well as the diculture legume-grass SH/PM had significantly fewer whitefly immatures compared with the weedy fallow control ( $F = 3.04$ ;  $df = 3, 6$ ;  $P = 0.0312$ ) (Table 4). None of the other treatments had significantly different whitefly numbers to the control.

**2007:** None of the treatments had fewer adult whiteflies than the control. Squash growing in plots treated with the grass monoculture PM and the diculture grass/legume SSG/VB had higher number of whiteflies than the weedy fallow (control) ( $F = 3.10$ ;  $df = 3, 6$ ;  $P = 0.0289$ ) (Table 3).

Overall, whitefly eggs in organic squash were much lower in 2007 than in 2006 (Table 4). The leaf disc counts revealed that organic squash plants

growing in monoculture plots where VB was incorporated into the soil had significantly higher number of whitefly eggs compared with all other treatments ( $F = 10.06$ ;  $df = 3, 6$ ;  $P < 0.0001$ ). Also, squash plants growing in monoculture plots treated with grass cover crop SSG, leguminous SH, and legume/grass dicultures SH/PM and SSG/VB had significantly lower number of whitefly eggs than the weedy fallow control ( $F = 10.06$ ;  $df = 3, 6$ ;  $P < 0.0001$ ) (Table 4). Egg counts in squash plots treated with PM were not significantly different to the control.

For whitefly immature counts on squash leaf discs, only plots treated with the monoculture grass PM and the diculture legume/grass SH/PM had higher numbers of whiteflies than the weedy control ( $F = 14.44$ ;  $df = 3, 6$ ;  $P < 0.0001$ ) (Table 4).

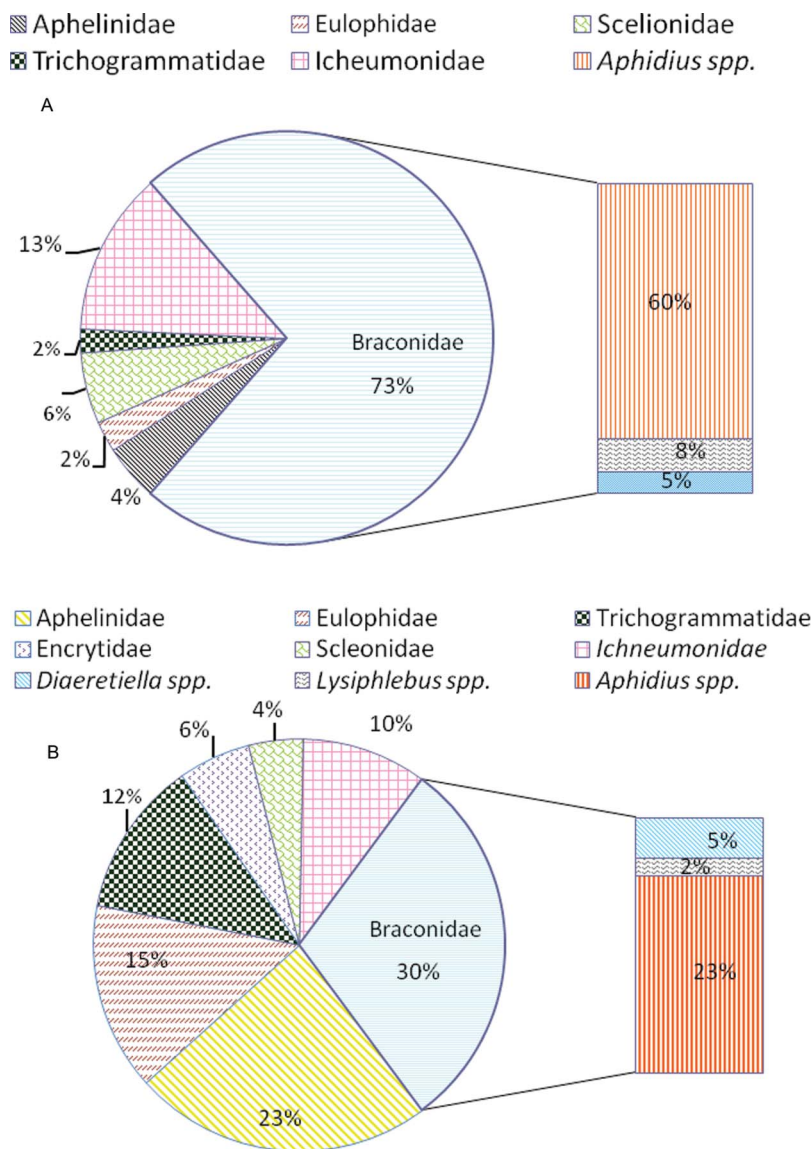
### Effects of Cover Crops on Natural Enemy Population in Organic Squash

Parasitoids that were captured during 2006 and 2007 included Aphelinidae: *Aphelinus* sp., *Encarsia* spp., Braconidae: *Aphidius* sp., *Chelonus* sp., and *Lysiphlebus testaceipes* (Cresson), *Diaeretiella* spp. Icheumonidae, Bethylidae, Scelionidae: *Telenomus* sp., Eucolidae, Mymaridae, Chalcididae, Eulophidae: Tetrastichinae, Trichogrammatidae, and Encyrtidae: *Metaphycus* sp., *Aphidius* spp. and (Figure 2). Several species of aphidophagous syrphid flies (Diptera: Syrphidae) were also captured among the squash plants included *Toxomerus* spp., *Platycheirus* spp. *Allograpta* spp. and *Sphaerophoria* spp. (Figure 3).

#### NATURAL ENEMIES ON SENTINEL SQUASH PLANTS AND IN PERIPHERAL AREAS

**2007 (Parasitoids):** Sentinel squash plants that were placed in each treatment immediately after cover crop was incorporated into the soil had no parasitoids. However, a few parasitoids were captured on yellow sticky traps from peripheral areas closely surrounding the treatment plots.

There were significant differences among habitats in the first and third week of sampling. During the first week, we recorded significantly more parasitoids in the grass/weeds area compared to the fruit tree region ( $F = 4.51$ ;  $df = 2, 4$ ;  $P = 0.0489$ ) (Table 5). The grass/weed areas had almost twice as many parasitoids as the fruit tree areas. However, by the end of the third week of sampling, there was no significant difference between the grass/weed area and the woods, but the woods had 1.5 times more parasitoids than the fruit tree region ( $F = 4.22$ ;  $df = 2, 4$ ;  $P = 0.0559$ ) (Table 5). The fluctuations in parasitoid numbers during each week were significant ( $F = 11.37$ ;  $2, 8$ ;  $P = 0.0046$ ) and there was also a significant interaction between the grass, woods and fruit trees (oranges) habitats and

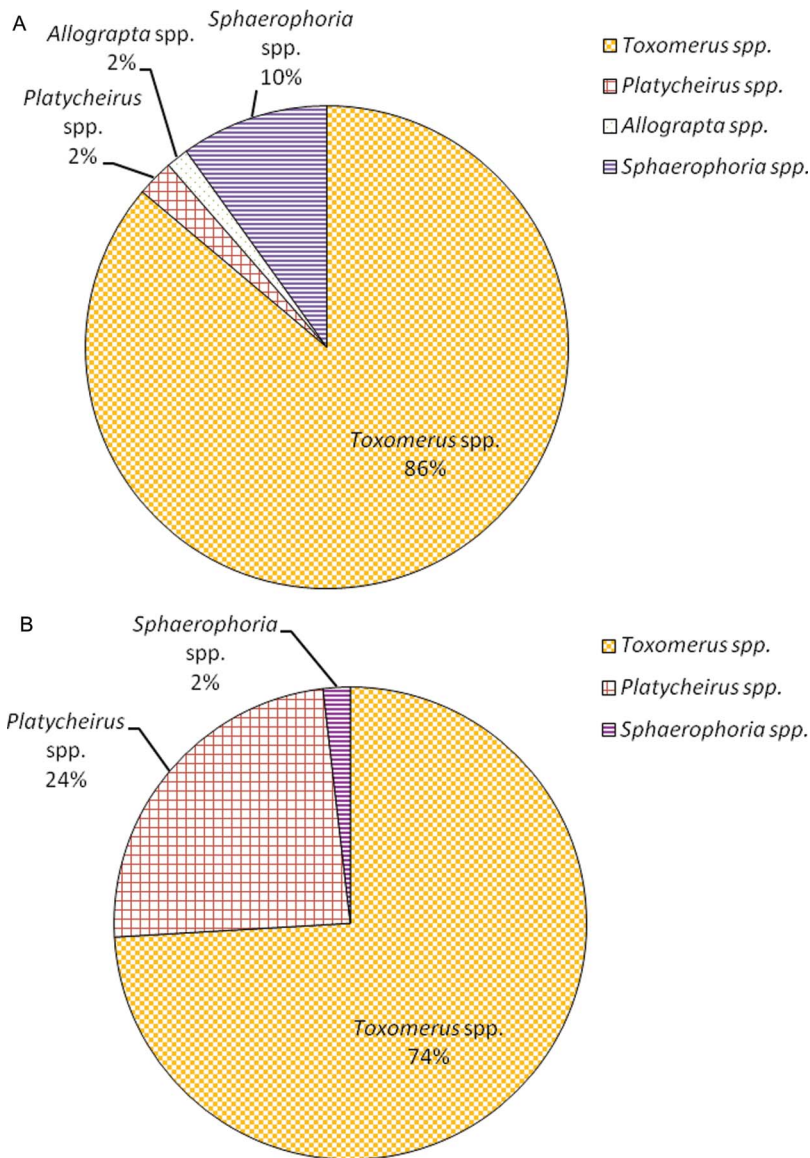


**FIGURE 2** Major parasitoid families in an organic squash field in A) 2006 and B) 2007 (color figure available online).

the weeks that sampling occurred (sampling periods) ( $F = 3.34$ ;  $df = 2, 24$ ;  $P = 0.0260$ ).

NATURAL ENEMIES ON ORGANIC SQUASH

**2006:** There was no significant difference in the number of spiders, parasitoids or ladybeetles in 2006 (Table 6). However, squash planted in



**FIGURE 3** Syrphid fly populations in an organic squash field in A) 2006 and B) 2007 (color figure available online).

areas where the SSG/VB mixture was incorporated in the soil had significantly higher number of syrphid flies than all the other treatments including the weedy fallow control ( $F = 6.48$ ;  $df = 3, 6$ ;  $P = 0.0009$ ) (Table 6). Monocultures SH, PM and the diculture SH/PM had significantly more syrphid flies than the control (Table 6).

During the first week of sampling, SSG and the SSG/VB mixture had significantly more *Delphastus pusillus* (LeConte) (Coleoptera:Coccinellidae)

**TABLE 5** Parasitoid population dynamics from peripheral habitats surrounding an organic field

Treatment	Mean $\pm$ SEM parasitoid numbers in 2007		
	Week 1 <sup>a</sup>	Week 2 <sup>b</sup>	Week 3 <sup>c</sup>
Grassy/weedy area	52.60 $\pm$ 11.46a	38.80 $\pm$ 6.74	16.00 $\pm$ 4.24ab
Fruit tree (oranges)	23.20 $\pm$ 4.39b	34.20 $\pm$ 6.68	8.00 $\pm$ 2.02b
Woods	41.00 $\pm$ 5.00ab	21.80 $\pm$ 1.82	25.80 $\pm$ 4.98a

Parasitoid data were square root transformed before analysis, but the means shown represent untransformed values. The letters a, b, or c refer to significantly different means. Means followed by the same letters are not significantly different ( $P = 0.05$  according to least square means test following repeated measures analysis, LS).

<sup>a</sup> $F = 4.51$ ;  $df = 2, 4$ ;  $P = 0.0489$ .

<sup>b</sup> $F = 1.72$ ;  $df = 2, 4$ ;  $P = 0.2396$ .

<sup>c</sup> $F = 4.22$ ;  $df = 3, 6$ ;  $P = 0.0559$ .

**TABLE 6** Mean  $\pm$  SEM number of natural enemies from in situ squash counts from cover crop treatments in 2006

Treatment	Mean $\pm$ SEM			
	Spiders <sup>a</sup>	Parasitoids <sup>b</sup>	Lady beetles <sup>c</sup>	Syrphid flies <sup>d</sup>
SH	0.00 $\pm$ 0.00	0.25 $\pm$ 0.25	0.00 $\pm$ 0.00	10.00 $\pm$ 1.77b
PM	1.00 $\pm$ 0.57	0.25 $\pm$ 0.25	0.00 $\pm$ 0.00	9.75 $\pm$ 1.54b
SH + PM	0.75 $\pm$ 0.25	0.25 $\pm$ 0.25	0.00 $\pm$ 0.00	9.25 $\pm$ 1.65b
SSG	0.00 $\pm$ 0.00	0.50 $\pm$ 0.28	0.50 $\pm$ 0.28	4.75 $\pm$ 1.54c
VB	0.75 $\pm$ 0.47	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	4.75 $\pm$ 1.37c
SSG + VB	0.00 $\pm$ 0.00	0.25 $\pm$ 0.25	0.25 $\pm$ 0.25	17.50 $\pm$ 3.71a
Weedy fallow (control)	0.00 $\pm$ 0.00	0.25 $\pm$ 0.25	0.00 $\pm$ 0.00	2.75 $\pm$ 0.85c

Beneficial arthropod data were square root transformed before analysis but means shown reflect untransformed data. The letters a, b, or c refer to significantly different means. Means followed by the same letters are not significantly different  $P = 0.05$  (LSD).

<sup>a</sup> $F = 2.49$ ;  $df = 3, 6$ ;  $P = 0.0620$ .

<sup>b</sup> $F = 0.43$ ;  $df = 3, 6$ ;  $P = 0.8503$ .

<sup>c</sup> $F = 2.05$ ;  $df = 3, 6$ ;  $P = 0.1108$ .

<sup>d</sup> $F = 6.48$ ;  $df = 3, 6$ ;  $P = 0.0009$ .

than all other treatments except PM ( $F = 2.96$ ,  $df = 3, 6$ ;  $P = 0.0346$ ) (Table 7). By the end of the sampling period, SSG and the SSG/VB mixture, had significantly more coccinellids ( $F = 8.97$ ;  $df = 3, 6$ ;  $P < 0.0001$ ) [Table 7] than most other treatments; however, PM was not significantly different than the SSG/VB treatment.

**2007:** The highest number of parasitoids were recorded in the SSG treatment compared with the weedy fallow ( $F = 11.01$ ;  $df = 3, 6$ ;  $P < 0.0001$ ). Squash plants in the SH treatment had the lowest numbers of parasitoids, but was not significantly different from PM, VB, or the control ( $F = 11.01$ ;  $df = 3, 6$ ;  $P < 0.001$ ) (Table 8). Aphid mummies were significantly higher on squash planted in areas where PM, VB and SSG/VB mixture were incorporated into the soil ( $F = 12.02$ ;  $df = 3, 6$ ;  $P < 0.0001$ ). Squash plants

**TABLE 7** *Delphastus pusillus* population dynamics in monoculture and diculture treatments in 2006

Treatment	Mean $\pm$ SEM whitefly predator numbers in 2006		
	Week 1 <sup>a</sup>	Week 2 <sup>b</sup>	Week 3 <sup>c</sup>
SH	0.75 $\pm$ 0.25b	0.25 $\pm$ 0.25c	0.00 $\pm$ 0.00c
PM	2.75 $\pm$ 1.75ab	2.25 $\pm$ 0.63c	2.75 $\pm$ 1.18bc
SH + PM	1.25 $\pm$ 0.63b	1.00 $\pm$ 0.40c	0.25 $\pm$ 0.25c
SSG	6.25 $\pm$ 2.42a	21.75 $\pm$ 4.95a	19.00 $\pm$ 4.91a
VB	0.25 $\pm$ 0.25b	0.50 $\pm$ 0.28c	0.50 $\pm$ 0.28c
SSG + VB	6.00 $\pm$ 2.27a	12.75 $\pm$ 5.00b	9.25 $\pm$ 0.25b
Weedy fallow (control)	0.50 $\pm$ 0.29b	0.75 $\pm$ 0.47c	1.25 $\pm$ 0.75c

Means for *Delphastus pusillus* data are untransformed values. The letters a, b, or c refer to significantly different means. Means followed by the same letters are not significantly different ( $P = 0.05$  according to least square means test following repeated measures analysis, LS).

<sup>a</sup> $F = 2.96$ ;  $df = 3, 6$ ;  $P = 0.0346$ .

<sup>b</sup> $F = 11.01$ ;  $df = 3, 6$ ;  $P < 0.0001$ .

<sup>c</sup> $F = 8.97$ ;  $df = 3, 6$ ;  $P = 0.0001$ .

**TABLE 8** Mean  $\pm$  SEM number of natural enemies from in situ squash leaf counts from cover crop treatments in 2007

Treatment	Mean $\pm$ SEM			
	Spiders <sup>a</sup>	Parasitoids <sup>b</sup>	Aphid mummy <sup>c</sup>	Syrphid flies <sup>d</sup>
SH	1.00 $\pm$ 0.57	2.00 $\pm$ 1.15c	10.75 $\pm$ 1.31b	1.25 $\pm$ 0.63b
PM	0.75 $\pm$ 0.47	4.25 $\pm$ 0.75bc	18.75 $\pm$ 3.94a	3.00 $\pm$ 0.91ab
SH + PM	0.25 $\pm$ 0.25	6.75 $\pm$ 1.88b	9.25 $\pm$ 2.25bc	1.50 $\pm$ 0.28b
SSG	0.25 $\pm$ 0.25	15.50 $\pm$ 0.64a	4.75 $\pm$ 2.83bc	2.00 $\pm$ 0.81b
VB	0.25 $\pm$ 0.25	3.77 $\pm$ 1.65bc	22.50 $\pm$ 2.95a	5.00 $\pm$ 0.82a
SSG + VB	0.25 $\pm$ 0.25	6.50 $\pm$ 2.06b	22.25 $\pm$ 2.13a	2.00 $\pm$ 0.41b
Weedy fallow (control)	0.25 $\pm$ 0.25	4.75 $\pm$ 1.70bc	3.55 $\pm$ 1.55c	1.25 $\pm$ 0.63b

Beneficial arthropod data were square root transformed before analysis but means shown reflect untransformed data. The letters a, b, or c refer to significantly different means. Means followed by the same letters are not significantly different  $P = 0.05$  (LSD).

<sup>a</sup> $F = 0.89$ ;  $df = 3, 6$ ;  $P = 0.5213$ .

<sup>b</sup> $F = 11.01$ ;  $df = 3, 6$ ;  $P < 0.0001$ .

<sup>c</sup> $F = 12.02$ ;  $df = 3, 6$ ;  $P < 0.0001$ .

<sup>d</sup> $F = 3.50$ ;  $df = 3, 6$ ;  $P = 0.0180$ .

from the weedy fallow control treatment had the lowest number of aphid mummies, but these were not significantly different from those plants growing in areas where SH/PM mixture or SSG was incorporated into the soil (Table 8).

In the first and second week of sampling, there were no significant differences in the number of *D. pusillus* among the treatments. However, in the third week of sampling SSG had significantly higher number of *D. pusillus* compared with all other treatments ( $F = 2.79$ ;  $df = 3, 6$ ;  $P = 0.0425$ ) (Table 9). The number of whitefly predators in 2007 was 10 times lower than in 2006. Treatment differences were observed in third week of sampling.



**TABLE 9** *Delphastus pusillus* population dynamics in monoculture and diculture treatments in 2007

Treatment	Mean $\pm$ SEM whitefly predator numbers in 2007		
	Week 1 <sup>a</sup>	Week 2 <sup>b</sup>	Week 3 <sup>c</sup>
SH	0.00 $\pm$ 0.00	0.25 $\pm$ 0.25	0.00 $\pm$ 0.00b
PM	0.50 $\pm$ 0.28	0.25 $\pm$ 0.25	0.25 $\pm$ 0.25b
SH + PM	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.25 $\pm$ 1.25b
SSG	1.25 $\pm$ 0.47	0.00 $\pm$ 0.00	2.75 $\pm$ 0.47a
VB	0.25 $\pm$ 0.25	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00b
SSG + VB	1.00 $\pm$ 0.71	0.00 $\pm$ 0.00	0.75 $\pm$ 0.47b
Weedy fallow (control)	0.50 $\pm$ 0.29	0.25 $\pm$ 0.25	0.50 $\pm$ 0.28b

*Delphastus pusillus* data were square root transformed before analysis, but the means shown represent untransformed values. The letters a and b refer to significantly different means. Means followed by the same letters are not significantly different ( $P = 0.05$  according to least square means test following repeated measures analysis, LS).

<sup>a</sup> $F = 1.53$ ;  $df = 3, 6$ ;  $P = 0.2248$ .

<sup>b</sup> $F = 0.69$ ;  $df = 3, 6$ ;  $P = 0.6589$ .

<sup>c</sup> $F = 2.79$ ;  $df = 3, 6$ ;  $P = 0.042$ .

### Squash Silver Leaf Disorder Rating

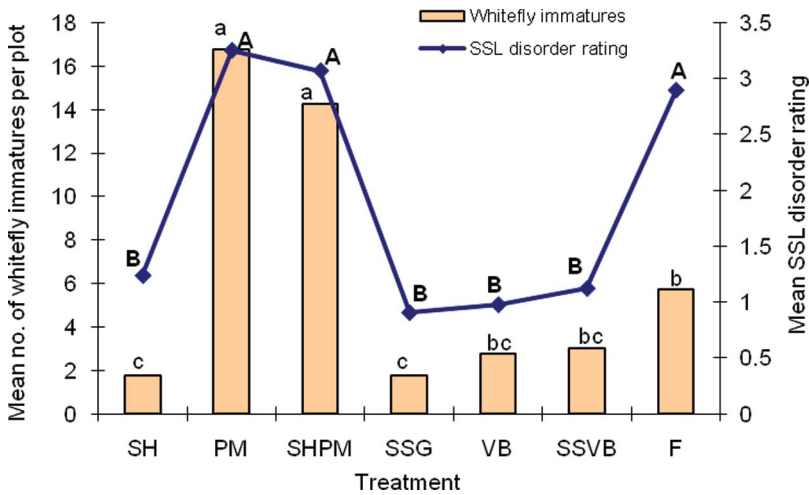
The rating for SSL disorder was highest on squash plants growing in areas where PM, SH/PM and weedy fallow vegetation was incorporated into the soil ( $F = 21.82$ ;  $df = 3, 6$ ;  $P < 0.0001$ ). The SSL ratings with all other treatments did not differ significantly (Figure 4). Overall, the SSL disorder ratings increased with the number of whitefly immatures. There was a significant and fairly strong ( $P = 0.0081$ ,  $R^2 = 0.78$ ) correlation between whitefly abundance and SSL disorder rating (Figure 5). When squash silver leaf (SSL) disorder was observed, SSG and SH monocultures had the lowest number of whiteflies immatures and significantly lower SSL ratings compared with the weedy fallow.

## DISCUSSION

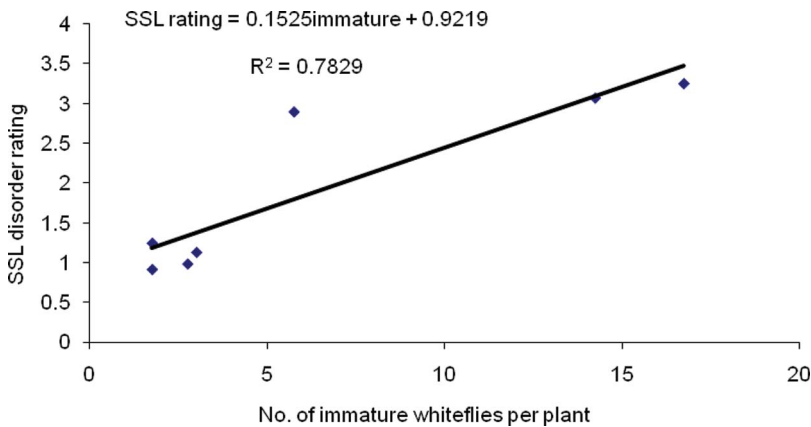
### Aphid and Whitefly Populations in Sentinel Plants, Peripheral Areas, and Organic Squash

#### APHIDS

To our knowledge, this is the first article that provides some evidence that pests and natural enemies (beneficials) from incorporated cover crops in organic systems can find refuge and survive on peripheral host plants and later invade subsequent cash crops. The high numbers of parasitoids in the grass/weed areas during the first week of sampling (Table 5) may indicate movement of some parasitoids from cover crops to adjacent vegetation. This vegetation may have provided sufficient resources to maintain



**FIGURE 4** Populations of whitefly immatures with squash silver leaf disorder rating in 2007 (color figure available online).



**FIGURE 5** Relationship of immature whiteflies with squash silverleaf disorder rating (color figure available online).

parasitoid numbers and allow for reestablishment in subsequent cash crop (squash).

The highest number of aphids was recorded in the wooded area. It is possible that there were more hosts for aphids to alight on in this area compared with the fruit tree or grassy areas. We noted that the wooded area consisted of a number of herbaceous plants and weeds that may have harbored more aphids to alight on.

Organic squash was grown for the first time on this site in 2006. In 2007 aphid mean averages per treatment were higher than in 2006. Control plots were void of cover crop treatments and squash plants in these

plots were not as vigorous as those in other plots in 2007. Consequently, the number of aphids in the control plots was low compared with other plots treated with cover crops.

In 2006, parasitoid numbers varied between 0 and 0.5; whereas in 2007 parasitoid numbers increased to as high as  $15.5 \pm 0.6$  in SSG plots (Tables 6 and 7). Overall, more aphid parasitoids including *Apbelinus* sp. (Aphelinidae), Braconidae: *Aphidius* sp., *Chelonus* sp., and *Lysiphlebus tescapipes* (Cresson) were recorded in squash plots during 2007. Also, in 2006 there were relatively no aphid mummies observed on squash plants, but in 2007 aphid mummies were abundant and relatively high numbers were recorded in squash plots treated with PM, VB and SSG/VB cover crops. The higher number of parasitoids may be the result of higher aphid densities observed in 2007. However, these high aphid densities did not appear to be regulated in plots where parasitoid numbers were significantly higher than the control (imperfectly density dependent factor; Pedigo 2009).

#### WHITEFLIES

Similar to aphids, the highest population of whiteflies was recorded in the wooded area, which may be related to more resources (host and egg laying habitats) available for whiteflies. As noted previously, the wooded area was inundated with weeds and herbaceous plants, which may have provided additional host and egg laying habitats for whiteflies.

In 2006, immature and adult whiteflies were significantly lower in PM, SH/PM, and SSG plots compared with the control. Overall, with the exception of PM and SH/PM, whitefly immatures and adults were lower in 2007 compared with 2006. In 2007, the number of immature and adult whiteflies in the control were low and was not significantly different from the other treatments except PM and SH/PM (2006–2007) and SSG/VB (2007) which had higher numbers. An interesting observation was that two of the plots (SH/PM and SSG) that had lower numbers of whitefly immatures in 2006 had significantly lower number of eggs in 2007. Eggs will eventually give rise to nymphs and adults potentially showing the same trend as 2006.

High numbers of whitefly immatures in PM and SH/PM corresponded to high incidences of SSL disorder, which supported the theory that the immatures are responsible for the SSL disorder (Nyoike et al. 2008, Nyoike and Liburd 2010).

Predaceous whitefly coccinellids (*D. pusillus*), which were observed in the cover crop treatments, were not captured during the squash cash crop. Usually, these predaceous coccinellids require very high populations of whiteflies to maintain reproduction (Hoelmer et al. 1993). *Delphastus pusillus* larvae are known to consume approximately 167 eggs per day and up to 1000 eggs before pupating (Hoelmer et al. 1993). From these studies,

on average, there were not more than 120 whitefly eggs and immatures in 2006 and not more than 20 whitefly eggs and immatures in 2007. Therefore, there may not have been sufficient number of whiteflies to allow the predator to successfully reproduce and regulate whitefly population.

Some of the parasitoids from the family Aphelinidae (*Encarsia* spp.) were identified as potential whitefly natural enemies. It is unclear whether or not these parasitoids played any role in regulating whitefly populations.

There were no consistent trends observed between 2006 and 2007 for aphid and whitefly numbers. The reason for the inconsistent trends between both years is unclear. Observed differences in squash treatments may be related to a number of factors including the presence of allelochemicals and/or soil nutrient quality from previous cover crop treatments. These factors may suggest, but do not prove to be responsible for a reduction in aphid numbers since this trend was not observed during both years.

For instance, in 2006, both grass cover crops (PM and SSG) as well as the legume/grass diculture SSG/VB had lower aphid populations. It is possible that the presence of allelochemicals from grass cover crops (SSG) may have contributed to a reduction in aphid populations in these selected plots (Baerson et al. 2008). Some of these allelochemicals are believed to be defensive chemicals that play key roles in preventing herbaceous insects from feeding on plants (Pedigo and Rice 2009) or chemicals that suppress root-knot nematodes (*Meloidogyne* spp.), which may have allowed for greater squash resistance to other insect pests including aphids.

Second, grass cover crops (SSG and PM) are also known to reduce pest numbers by altering environmental conditions (change in soil nutrient composition). Grasses are known to be nitrogen scavengers and have high C:N ratios. For example, pea cultivars with low amino acids due to nitrogen deficiency and elevated sugar levels show resistance to the pea aphid *Acyrtosiphon pisum* (Harris) Auclair et al. 1957).

Alternatively, nitrogen levels are also known to affect whitefly populations. Athar et al. (2011) found that excessive doses of nitrogen can produce lush green plants that attract whiteflies (*B. tabaci* B-biotype) but as the plant's optimal nutritional quality decreases due to reduced nitrogen levels, the plant becomes even more susceptible to whitefly infestations.

## CONCLUSION

Overall, this paper provided some evidence that pests and natural enemies from incorporated cover crops are capable of invading cash crops and subsequently increase pest pressure and/ or provide protection for cash crop. The mechanisms for pest suppression is unclear but could be related to the presence of allelochemicals, soil nutrient quality and natural enemy complex. The level of pest suppression will, however, depend on the diversity

of the peripheral vegetation surrounding the field because alternate food sources need to be present to maintain natural enemy populations until the subsequent crop is established.

Although SSG plots had the highest number of whitefly coccinellids at the end of 2006 and 2007, and the highest number of parasitoids in 2007 compared with all other treatments, these high numbers of natural enemies may not translate into pest suppression. However, this monoculture (SSG) demonstrates potential and could be recommended as an agent for suppressing whiteflies on organic farms. The grass cover crop SSG and diculture SH/PM was also observed to have relatively low whitefly populations indicating increased potential for use in organic fields.

The high number of whitefly eggs recorded in plots treated with the monoculture VB was not surprising. Velvet bean, *M. pruriens*, is an excellent host for the whitefly, *B. tabaci* B-biotype. It is possible that a resident population of whiteflies existed in these plots that quickly invaded squash plots. Growers that are considering growing a crop such as squash that is susceptible to whiteflies should avoid using velvet bean as a cover crop as this could seriously increase whitefly numbers and devastate yield.

With respect to aphid management, squash planted in the diculture SSG/VB plots harbored high levels of syrphid flies (2006), which are effective aphid predators, and holds potential for consideration as a diculture treatment for crops where aphids are the key single pest. The grass monoculture PM was also observed to have low aphid populations. Pearl millet is also a potential cover crop for organic fields prone to aphid infestation.

It does not appear that the diculture SH/PM mixture is suitable for aphid suppression on organic farms. High levels of aphids were recorded in squash planted in this plot in both years.

These results can help to develop sustainable pest management practices for organic growers, but also shows exceptional promise for the development of future organic pest management programs.

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