Accepted: 9 September 2021

DOI: 10.1111/eea.13147

Entomologia Experimentalis et Applicata

ORIGINAL ARTICLE

Can the introduction of companion plants increase biological control services of key pests in organic squash?

Lorena Lopez[1] | **Oscar E. Liburd**[2]

[1]Department of Entomology, Eastern Shore Agricultural Research and Extension Center, Virginia Tech, Painter, Virginia, USA

[2]Department of Entomology and Nematology, University of Florida, Gainesville, Florida, USA

Correspondence
Lorena Lopez, Eastern Shore Agricultural Research and Extension Center, 33446 Research Drive, Painter, VA 23420, USA.
Email: lorelopezq257@vt.edu

Funding information
Florida Department of Agriculture and Consumer Services, Grant/Award Number: 02291; Southern SARE, Grant/Award Number: RD309-129/S000843

Abstract

Florida (USA) is a major producer of squash, *Cucurbita pepo* L. (Cucurbitaceae), with approximated 16% of the US production in 2019, valued at about 35 million USD. Major insect pests, including the sweetpotato whitefly MEAM1, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), and the melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), jeopardize plant development and transmit viruses of economic importance that can cause up to 50% yield loss in squash crops. Pesticides are generally used for insect management in squash, but the development of insecticide resistance and their non-target effects are major concerns. A combination of non-pesticidal approaches was evaluated, including intercropping flowering plants, augmentation, and conservation biological control to manage key pests in organic squash. Refugia increased natural enemies around the squash; however, only a few beneficial arthropods moved from the companion plants towards the squash plants. Whitefly densities and squash silverleaf ratings were reduced, whereas natural enemies were more abundant when the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) was released alone or together with sweet alyssum, *Lobularia maritima* (L.) Desv. (Brassicaceae). All companion plants used in this study increased natural enemies, but only African marigolds and sweet alyssum ultimately increased biological control activities.

KEYWORDS
marigold, cowpea, alyssum, predatory mite, refugia, intercropping, *Bemisia tabaci*, *Aphis gossypii*, augmentation, conservation biological control, *Amblyseius swirskii*, *Cucurbita pepo*

INTRODUCTION

The use of insecticides continues to be a common management tactic used by conventional growers of squash, *Cucurbita pepo* L. (Cucurbitaceae), against major insect pests such as the melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), and the sweetpotato whitefly MEAM1, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Nyoike & Liburd, 2010). However, total reliance on chemicals is not a sustainable pest management strategy due to the problems associated with pest resistance and its effects on non-target organisms (Razze et al., 2016a). Organic squash growers have limited pesticide options and frequently use preventative and cultural management techniques to reduce aphid and whitefly pressure (Razze et al., 2016a). Generally, the implementation of a single management tactic does not achieve acceptable levels of pest suppression; therefore, there is a constant need for multi-tactic management approaches to successfully suppress key pests in conventional and organic squash production. In Florida (USA), organic squash is grown during the fall and spring seasons. Both whiteflies and aphids can cause a significant amount of indirect injury throughout the year due to the viruses that they transmit, causing up to 50% yield loss in squash (VSCNews, 2020). Whitefly-transmitted viruses are easier to detect during the fall growing season when the whitefly pressure is higher (Mossler & Nesheim, 2011). Contrastingly, aphid-transmitted viruses can be observed in both seasons as the aphid population changes throughout the year (Mossler & Nesheim, 2011).

Aphids transmit most of the economically important viruses that infect squash, including *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV, all three Potyviridae), and *Cucumber mosaic virus* (CMV, Bromoviridae) (Nyoike & Liburd, 2010). Aphid-transmitted viruses are usually transmitted in a non-persistent manner. They are acquired within seconds with no latent period needed before transmitting the virus to healthy plants through probing or feeding.

Sweetpotato whiteflies transmit the *Cucurbit leaf crumple virus* (CuLCrV, Geminiviridae), *Squash vein yellowing virus* (SqVYV, Potyviridae), and *Cucurbit yellow stunting disorder virus* (CYSDV, Closteroviridae) (Akad et al., 2008; McAvoy, 2016; Razze et al., 2016a). Whiteflies need to feed on infected plants for several minutes to acquire the virus, and after a latent period that can last from minutes to hours, they can transmit the virus to healthy plants (Whitfield et al., 2015).

Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae) is an effective predator of the key pest and disease vector sweetpotato whitefly, and of secondary squash pests such as thrips (Xu & Enkegaard, 2010; Buitenhuis et al., 2015; Kutuk et al., 2016). It has been used in several vegetable crops including pepper, cucumber, and eggplant for management of insects and mite pests (Nomikou et al., 2002; Stansly & Castillo, 2009; Stansly & Natwick, 2010; Farkas et al., 2016). Calvo et al. (2011) demonstrated the effectiveness of *A. swirskii* in suppressing the sweetpotato whitefly and western flower thrips, *Frankliniella occidentalis* (Pergande), populations with up to 99% suppression in greenhouse-grown cucumber. The option of targeting two pests with a single natural enemy has positive implications for biological control and resembles pest-predator complexes in natural conditions (Messelink et al., 2010; Calvo et al., 2015). Because *A. swirskii* is a generalist predatory mite species it has the additional capacity to feed on pollen from various plants species (e.g., pepper, cattail, and others), increasing the chance of establishment at early stages of the crop when pests are absent but flowering plants are present (Calvo et al., 2015; Lopez et al., 2017). Despite its wide use in vegetable crops, most studies investigating the performance of *A. swirskii* have been conducted in greenhouse-grown vegetables (Stansly et al., 2018; Kheirodin et al., 2020) and very few have evaluated its performance and establishment in squash crops (Calvo et al., 2015).

Companion planting or intercropping involves growing other plants (crop or non-crop) within the cropping system together with the cash crop. It has been used as a diversification tactic mostly to increase soil quality, yield, and promote biocontrol services by beneficial arthropods (Wang, 2012; Juventia et al., 2021). Insectary plants are used in conservation biological control within vegetable production to provide alternative shelter and food items to beneficial arthropods (Badenes-Perez, 2019). Due to its deterrent effects on certain insect pests, African marigolds, *Tagetes erecta* L. (Asteraceae), have been used in several studies as companion plants, cover crops, and as insectary plants to enhance beneficial arthropods in vegetable cropping systems (El-Gindi et al., 2005; Jankowska et al., 2012; Wang, 2012). Field studies in onion, tomato, and eggplant crops have shown that intercropping using marigold plants contributes to insect pest management by increasing the density of natural enemies present in the crop canopy (Silveira et al., 2009; Jankowska et al., 2012).

Cowpea, *Vigna unguiculata* (L.) Walp. (Fabaceae), has also shown potential as a companion plant due to its extrafloral nectaries that attract beneficial arthropods including hoverflies, parasitoids, lady beetles, minute pirate bugs, and ground beetles (Letourneau, 1990; Wang, 2012; Koptur et al., 2018). Similarly, the perennial herb sweet alyssum, *Lobularia maritima* (L.) Desv. (Brassicaceae), has been largely investigated as a cost-effective insectary plant for attracting aphid predators and parasitoids, as well as pollinators into the cropping systems (Bugg et al., 2008; Gillespie et al., 2011; Hogg et al., 2011; Skirvin et al., 2011; Brennan, 2013; Gontijo et al., 2013; Tanga & Niba, 2019). Unlike other insectary plants, sweet alyssum's blooming period is longer, attracts fewer bees that can outcompete hoverflies, and has been related to reductions in whitefly populations due to its attractiveness to generalist predators (Badenes-Perez, 2019).

Squash is considered an excellent candidate for intercropping systems due to its short production cycle (approximately 8 weeks) and ease of growing. In addition, biocontrol agents can be released within squash crops to enhance biocontrol services. Thus, the goal of this study was to evaluate the potential of three companion plants as a technique for conservation biological control alone and in combination with the release of a generalist predator (*A. swirskii*) as an augmentative biocontrol technique to determine whether there are increases in biocontrol services against key pests in squash. Furthermore, we determined the potential of companion plants as refugia for pests and natural enemies, compared the use of these combined techniques with commonly used insecticides that are labelled for organic use to suppress aphids and whiteflies. Finally, we evaluated the effect of companion plants and *A. swirskii* on viral incidence and yield in organic zucchini squash.

We predict that companion plants will serve as refugia by offering shelter, oviposition sites, and alternative food sources to support naturally occurring and released (*A. swirskii*) biocontrol agents in times of prey (pest) scarcity. Furthermore, we expect that whereas parasitoids and larger predators attracted by the non-crop plants – such as syrphids, big eyed bugs, and *Orius* spp. – will feed on aphids, *A. swirskii* will seek out sweetpotato whiteflies and will establish early in the season by feeding on pollen from the companion plants or alternative prey such as thrips.

MATERIALS AND METHODS

Study site

Two year-round experiments were conducted in 2015 and 2017 at the University of Florida's Plant Science Research and Education Unit (PSREU), Citra, FL, USA (29°24'28.0"N, 82°08'50.1"W). Each year of experiments comprised two squash seasons, fall and spring. In North-Florida, the spring season starts by planting squash from late February to early March, and the season lasts until late May. The fall season usually starts in mid-September and lasts until early or mid-November. A 0.24-ha experimental area was used in 2015, and a 0.35-ha area was designated for 2017 experiments.

Plant culture

Zucchini squash cultivar 'Cash Flow' (Siegers Seed, LaBelle, FL, USA) was used as the crop in all experiments. In 2015, African marigold cultivar 'Crackerjack' (Stokes Seeds, Buffalo, NY, USA) and cowpea cultivar 'Mississippi Silver' (Urban Farmer, Westfield, IN, USA) were used as companion plants. In 2017, African marigold was re-used, and cowpea was replaced with sweet alyssum cultivar 'Tall White' (Urban Farmer).

Squash plants were sown directly in the field in double rows at 35-cm intervals. To synchronize the maturity periods of all plant species, companion plants were grown from seeds in the greenhouse 3–4 weeks before planting the squash. In the field, plants were drip irrigated and fertigated weekly after germination using a 6–0–8 micro blend fertilizer (Mayo, Lake City, FL, USA). A rotation of the organic fungicides Regalia (Marrone Bio Innovations, Davis, CA, USA) and DoubleNickel55 (Certis USA, Columbia, MD, USA) was used weekly on the squash against downy and powdery mildew.

Experimental design

In 2015, a randomized complete block design with five treatments and four replications was used to evaluate the effect of companion plant species (separate or marigold and cowpea mixed) on the establishment of naturally occurring beneficial arthropods with potential to suppress insect pests in organic squash. Experimental plots were 6 × 4.4 m, separated by 7 m of bare soil (buffer zone) on all sides. Each plot comprised three raised beds covered with black plastic (18 cm high, 91 cm wide,

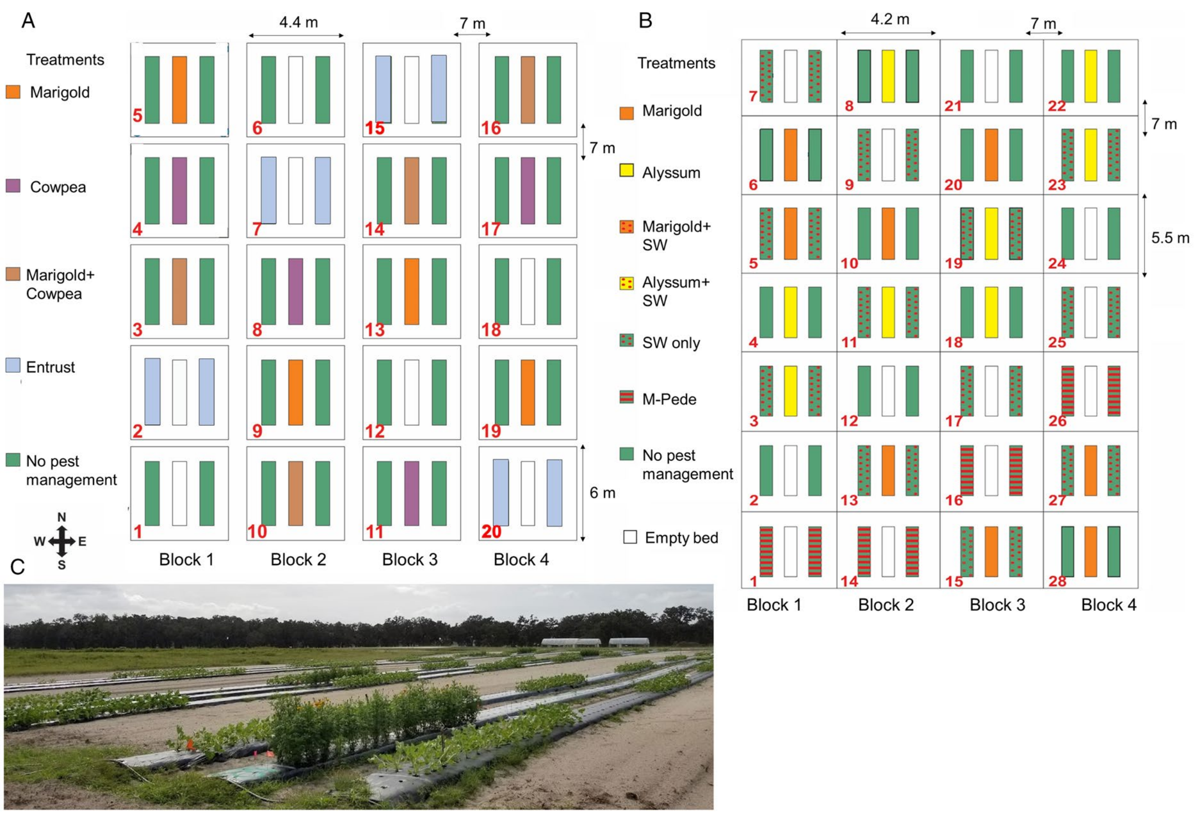

FIGURE 1 Experimental layout used for the experiments in (A) 2015, (B) 2017, and (C) field in 2015. The insecticide Entrust was used in 2015 and replaced in 2017 by M-Pede. *Amblyseius swirskii* (SW) was only released in 2017 experiments

1.06 m apart) (Figure 1A). Plants were sown in double rows of 22 plants each. Treatments were defined by the species of companion plant intercropped in the middle row and the type of pesticides used. Treatments included: (1) marigold; (2) cowpea; (3) marigold and cowpea mixed; (4) no companion plant but the use of spinosad (Entrust; Dow AgroSciences, Indianapolis, IN, USA) for insect control; and (5) no companion plant or any type of pest management (control). Entrust was applied twice to the squash plants in the selected treatments.

Modifications were made in 2017 to optimize treatment effectiveness and efficiency, based on the results obtained in 2015. Data collected in 2015 showed that Entrust had no suppressive effect on whitefly populations; therefore, M-Pede (Gowan Company, Yuma, AZ, USA), an insecticide with potassium salts of fatty acids as active ingredient, was used during the 2017 experiments (Razze et al., 2016b). Additionally, releases of the predatory mite *A. swirskii* were included in 2017 as a complementary tactic with the use of companion plants.

In 2017, the experimental design was a randomized complete block with seven treatments and four replications. Plot size was 5.5 × 4.2 m with buffer zones of 7 m on all sides. Each plot comprised three raised beds (18 cm high, 91 cm wide, 1.06 m apart). Plants were sown in double rows of 18 plants each. Treatments were defined by the species of companion plant intercropped in the middle row and the presence or absence of predatory mites as follows: (1) marigold; (2) sweet alyssum; (3) marigold and *A. swirskii* released on the squash; (4) sweet alyssum and *A. swirskii* released on the squash; (5) no companion plant only *A. swirskii* released on the squash; (6) no companion plant or *A. swirskii*, only the use of M-Pede for insect management; and (7) no companion plant or any type of pest management (control) (Figure 1B). This type of treatment arrangement helped to determine whether companion plants – marigold or sweet alyssum – intercropped in organic squash will give additional benefits in terms of pest suppression compared with augmentative releases of *A. swirskii*, and how this would compare with the periodic application of a pesticide (M-Pede) labelled for organic use. M-Pede was applied twice to the squash in the selected treatments.

Predatory mites

Amblyseius swirskii mites were purchased in 500-ml bottle shaker formulation (Koppert Biological Systems, Howell, MI, USA) with vermiculite as bran carrier. Five bran samples (0.5 ml each) were checked under the dissecting microscope to confirm that the predatory mites were active prior to release. *Amblyseius swirskii* was released in the field on the day of arrival by scattering the bran on top of the squash foliage. Each bed (ca. 5 m^2) containing squash plants was treated with approximately 20 ml of bran per bed 3 weeks after planting the squash. Approximately 250 *A. swirskii* motiles were released per m^2. The release rate used was based on the rate recommended for high pest infestations (100–300 mites m^{-2}; Koppert Biological Systems, 2022).

Sampling

Beneficial arthropods (predators and parasitoids), pests including aphids and sweetpotato whiteflies, and silverleaf disorder were monitored in each plot and recorded for the squash as described below. Sampling was conducted weekly during a 5-week period each season starting 3 weeks after planting (WAP). Viral disease incidence was screened once during the last 3 weeks of each squash season before marketable yield was recorded. Beneficial arthropods and pests were also recorded in the companion plants weekly for 5 weeks following the same sampling protocol used in the squash.

All collected samples were processed at the Small Fruit and Vegetable IPM Laboratory at the University of Florida (Gainesville, FL, USA).

Beneficial arthropods

Beneficial arthropod species including predators and parasitoids were recorded weekly using in situ counts from six squash and six companion plants (chosen haphazardly) per plot in 2015, and four squash and three companion plants per plot in 2017. The leaf-turn method was used and consisted of gently turning over three leaves per plant and counting the beneficial arthropods observed (Nyoike & Liburd, 2010). In addition, parasitoids and predators were monitored weekly using three 28 × 23-cm yellow sticky traps (Great Lakes IPM, Vestaburg, MI, USA) per plot in 2015 and two sticky traps per plot in 2017. Sticky traps were left in the field for 48 h, traps were collected in ziplock bags and processed in the laboratory. A representative sample from each species was collected and mounted for identification.

In 2017, *A. swirskii* was monitored weekly by collecting three leaves from four squash plants (48 leaves per treatment). One 4-cm-diameter leaf disc was taken from each leaf using a cork borer (Cole-Parmer, Vernon Hills, IL, USA) in the laboratory and the numbers of *A. swirskii* eggs and motiles (nymphs, adult males, and females) per leaf were recorded. Additionally, three leaves from three companion plants were excised and brought back to the laboratory to monitor movement of predatory mites from the squash to the neighboring flowering companion plants.

Aphids

As described above, the leaf turn method was used to measure the population of winged and wingless aphids. These were sampled from six squash and six companion plants per plot in 2015. In 2017, four squash and three companion plants per plot were sampled. Winged aphids were also monitored using two clear pan traps (PackerWare, Lawrence, KS, USA) per plot in 2015 and one pan trap per plot in 2017 experiments. Each pan trap contained approximately 250 ml of 5% detergent solution (Colgate-Palmolive, New York, NY, USA). The detergent solution was refilled, collected, and transported weekly to the laboratory for counting and identification.

Whiteflies

Adult whiteflies were monitored weekly in the yellow sticky traps used for beneficial arthropod monitoring. The number of adult whiteflies per trap was recorded. In 2015, immature whiteflies were monitored weekly by collecting three leaves from six squash plants (72 leaves per treatment) and producing leaf discs for examination. Leaf discs collected for sampling of *A. swirskii* in the 2017 experiments were also examined under a dissecting microscope for immature whiteflies in the 2017 experiments.

Silverleaf disorder and viral diseases

One young leaf was excised from two squash plants per plot, for a total of 40 and 48 leaves collected in 2015 and 2017, respectively. Samples were collected 1 week before squash termination, transported to the laboratory in a cooler, and then stored at −80 °C until processed. Only samples from the fall season were assayed because of low whitefly abundances and low disease incidence during the spring growing season. Leaf samples were assayed for four aphid-transmitted cucurbit viruses (PRSV, WMV, ZYMV, and CMV) and one whitefly-transmitted virus (CuLCrV). Double or triple antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA or TAS-ELISA) was conducted for aphid-transmitted viruses and PCR for CuLCrV (Nyoike et al., 2008).

Reagent sets for ELISA assays were obtained from Agdia (Elkhart, IN, USA), as well as positive and negative controls to guarantee assay reliability. Reagent kits for each *Potyvirus* were used in 2015 and one reagent kit for the *Potyvirus* group was used in 2017. The substrate (p-nitrophenyl phosphate, PNPP) absorbance (optical density) was measured at 405-nm wavelength using a spectrophotometer to estimate virus concentration. Four times the mean plus the standard deviation of the negative control absorbance was used as a cut-off value to distinguish virus presence (>cuff-off value) from absence (<cuff-off value).

To conduct PCR, liquid nitrogen was used for sample disruption of ca. 0.2 g of plant tissue per sample and DNeasy Plant Mini Kits (Qiagen, Germantown, MD, USA) were used for DNA extraction. Apex Hot Start 2× blue master mix (Genesee Scientific, San Diego, CA, USA) and CuLCrV-specific primers from the DNA-B component (V1324, 5′-TTCTTCTGGTAAAATATGGC-3′ and C2370, 5′-CGACGAGATATGTCAACG-3′; Hagen et al., 2008) were obtained from Integrated DNA Technologies (Coralville, IA, USA) to direct the amplification of an expected ca. 1-kb fragment from the sample tissue. PCR-amplified DNA was separated by electrophoresis and visualized under UV light. Amplicons were sequenced to confirm CuLCrV presence.

Additionally, squash silverleaf (SSL) disorder caused by the feeding of the immature stages of sweetpotato whiteflies was monitored weekly by randomly selecting six squash plants per plot in both years and scoring them with an arbitrary index as follows: 0 = asymptomatic, 1 = young leaves with secondary veins silver, 2 = leaves with veins pale and appearing 'netted', 3 = leaves with primary and secondary veins silvering, 4 = silvering extends between veins, and 5 = various leaves with complete silvering.

Yield

Total marketable yield was estimated by harvesting and weighing the squash from all plants in the field twice per week during the last 3–4 weeks. Fruit was categorized as marketable by examining the fruit and finding no evidence of viral symptoms or injuries. Fruit with irregular ripening or viral symptoms was weighed separately and categorized as unmarketable. Fruit with borrowing signs from pickleworms, *Diaphania nitidalis* Stoll (Lepidoptera: Crambidae), were weighed separately. Total marketable and unmarketable yield, and total fruit injured by pickleworms were compared among treatments (Figure 1C).

Statistical analysis

In both 2015 and 2017, repeated measures analysis was performed to determine the effect of companion plant species alone, mixed, or its combination with *A. swirskii* on beneficial arthropod and insect pest abundance. All response variables were fitted by either a generalized linear mixed model (GLMM) or a linear mixed model (LMM).

The numbers of beneficial arthropod or insect pest per plot, including *A. swirskii* eggs and motiles, recorded by leaf collection, in situ counts, pan traps, and sticky traps, were fitted using a GLMM. The GLIMMIX procedure was implemented following either a Poisson distribution with Laplace adjustment or a negative binomial distribution to correct over-dispersion when needed. This model considered the fixed-effect factors of treatment, time (weeks), and their interaction. In addition, random effects of block and block within time were considered. The repeated measurements were considered by including a random factor of plot, corresponding to a compound symmetry structure.

Averaged SSL indexes and squash yields per plot were compared among treatments by using the MIXED procedure and degrees of freedom were adjusted using the Kenward-Rogers correction. No transformation was used for these variables. The LMM considered the fixed effect factors of treatment, time, and their interaction, together with a random effect of block. The repeated measurements were modeled using an autoregressive error structure of order 1 for each plot.

Comparisons of means among treatments for both GLMM and LMM were obtained by requesting LSMEANS from each procedure and the SLICE function for the effect of treatment when the GLMM was implemented. Data from squash plants and companion plants were analyzed separately. For all tests, $\alpha = 0.05$. All models were fitted using SAS v.9.4 (SAS Institute, Cary, NC, USA). Descriptive statistics were used to compare viral incidence among treatments due to the low viral incidence.

RESULTS

In total, 147 insect morphospecies from 64 families in the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Odonata, Orthoptera, and Thysanoptera were collected from the experiments in 2015 and 2017. Parasitoid wasps and predators accounted for 36% (53) and 20% (29) of the species, respectively. Important parasitoid families included Platygastridae, Trichogrammatidae, Encyrtidae, Pteromalidae, Aphelinidae, and Dryinidae. Important predatory species included minute pirate bugs (*Orius* sp.), big-eyed bugs (*Geocoris* sp.), lady beetles, and the introduced predatory mite *A. swirskii*.

Plant-feeding species accounted for 34% (50) of the species, but only eight of them were pests of concern in squash crops including the melon aphid, sweetpotato whiteflies, thrips, melonworms (*Diaphania hyalinata* L.), and pickleworms (*D. nitidalis*). The remaining 10% (15) of the species were identified as polyphagous species with no apparent harming potential to the squash.

Naturally occurring predators

Most beneficial arthropods were collected on yellow sticky cards except for *Orius* species. Marigolds served as a host for *Orius* sp., as adults and immature stages of this predator were found developing in the plants in 2015 and 2017. In spring 2015, the numbers of minute pirate bugs collected in the yellow sticky cards differed by treatment ($F_{4,12} = 25.64$, $P<0.001$), with no time or interaction effect. Approximately 2× fewer *Orius* sp. were collected in the treatment with no pest management compared with treatments that included companion plants (Table 1). When conducting in situ counts, the numbers of *Orius* sp. found in the companion plants differed by treatment ($F_{2,6} = 16.56$, $P = 0.003$), with no time or interaction effect. The *Orius* sp. found in the cowpeas planted alone (mean ± SE = 2.44 ± 0.81 individuals per plant) were significantly lower compared to the other treatments including companion plants (16.35 ± 2.7 and 15.86 ± 2.64 individuals per plant for marigolds alone and marigolds mixed with cowpeas, respectively). Low minute pirate bug numbers (0.19 ± 0.05 individuals per trap) were recorded in the yellow sticky traps and during in situ counts in fall 2015 (data not included in tables).

In spring 2017, the numbers of minute pirate bugs collected in yellow sticky traps differed by treatment ($F_{6,18} = 4.34$, $P = 0.007$), with time effect ($F_{4,84} = 4.98$, $P<0.001$), and no interaction effect. Fewer minute pirate bugs were recorded in the treatment with no pest management and the treatment with marigolds as companion plants together with *A. swirskii* release compared with other treatments (Table 2). Similarly, the number of minute pirate bugs recorded in the companion plants using in situ counts in spring 2017 differed by treatment ($F_{3,9} = 18.60$, $P = 0.0003$), with time ($F_{4,12} = 23.87$, $P<0.0001$), and interaction effect ($F_{10,116} = 12.83$, $P<0.0001$). The numbers of *Orius* sp. recorded in the marigolds alone or together with predatory mites were 5–6× higher (15.7 ± 3.28 and 13.08 ± 2.82 individuals per plant, respectively) compared with the numbers recorded in the alyssum alone or together with predatory mites (3.09 ± 1 and 1.66 ± 0.68 individuals per plant, respectively). No minute pirate bugs were collected during the fall of 2017.

Cowpeas were attractive to predatory species, especially adult and immature stages of coccinellids. In spring 2015, the numbers of coccinellids collected in the yellow sticky traps differed by treatment ($F_{4,12} = 9.68$, $P = 0.0009$), with no time or interaction effect. The number of coccinellids were similar among treatments except for the treatment including Entrust applications that showed the lowest number of lady beetles.

Low numbers of coccinellids were recorded during the fall of 2015 with no significant differences among

TABLE 1 Mean (± SE) number of predators and parasitoids collected per yellow sticky trap over a 5-week period during the 2015 experiments. Back-transformed data are shown

Season	Treatment/family	Marigold	Cowpea	Marigold+cowpea	Entrust (spinosad)	No pest management
Spring	Anthocoridae (minute pirate bugs)	3.04 ± 0.24b	3.76 ± 0.28a	3.21 ± 0.25ab	1.77 ± 0.17c	1.44 ± 0.15c
	Coccinellidae (lady beetles)	1.69 ± 0.31a	1.74 ± 0.32a	2.06 ± 0.37a	0.89 ± 0.18b	2.06 ± 0.37a
	Dolichopodidae (long-legged flies)	16.84 ± 1.59b	16.68 ± 1.57b	18.70 ± 1.75a	15.42 ± 1.46b	16.13 ± 1.52b
	Parasitoids (Hymenoptera)	10.17 ± 1.04b	10.92 ± 1.11ab	12.05 ± 1.22a	6.64 ± 0.70c	7.07 ± 0.74c
Fall	Coccinellidae (lady beetles)	0.31 ± 0.09	0.50 ± 0.12	0.31 ± 0.09	0.31 ± 0.09	0.25 ± 0.07
	Dolichopodidae (long-legged flies)	3.23 ± 0.33c	3.89 ± 0.37bc	3.49 ± 0.35bc	6.07 ± 0.53a	4.15 ± 0.39b
	Parasitoids (Hymenoptera)	18.50 ± 4.93bc	16.07 ± 4.29d	19.28 ± 5.14b	21.78 ± 5.81a	18.07 ± 4.82c

Means within a row followed by the same letter are not significantly different (Lsmeans: P>0.05).

TABLE 2 Mean (± SE) number of predators and parasitoids collected per yellow sticky trap over a 5-week period during the 2017 experiments. Back-transformed data are shown

Season	Treatment/family	Marigold	Alyssum	Marigold + *A. swirskii*	Alyssum + *A. swirskii*	*A. swirskii* only	M-Pede	No pest management
Spring	Anthocoridae (minute pirate bugs)	0.7 ± 0.13a	0.7 ± 0.13a	0.3 ± 0.08b	0.99 ± 0.15a	0.7 ± 0.13a	0.7 ± 0.13a	0.2 ± 0.07b
	Coccinellidae (lady beetles)	1.05 ± 0.22	0.76 ± 0.17	1.24 ± 0.25	1.53 ± 0.29	1.05 ± 0.22	0.95 ± 0.20	0.95 ± 0.20
	Dolichopodidae (long-legged flies)	14.50 ± 1a	14.01 ± 0.97ab	11.62 ± 0.83cd	12.91 ± 0.91abc	10.23 ± 0.75de	12.72 ± 0.89bc	8.84 ± 0.89e
	Parasitoids (Hymenoptera)	10.52 ± 1.56bc	11.02 ± 1.62ab	13.41 ± 1.95a	7.56 ± 1.14e	8.42 ± 1.27d	7.55 ± 1.14e	9.03 ± 1.34cd
Fall	Dolichopodidae (long-legged flies)	18.33 ± 1.40a	14.66 ± 1.15bc	17.93 ± 1.37a	13.87 ± 1.10c	8.52 ± 0.73e	16.44 ± 1.27ab	11.89 ± 0.96d
	Parasitoids (Hymenoptera)	25.73 ± 3.49	27.29 ± 3.70	29.11 ± 3.93	25.85 ± 3.50	23.76 ± 3.23	32.85 ± 4.43	25.60 ± 3.47

Means within a row followed by the same letter are not significantly different (Lsmeans: P>0.05).

treatments and no time or interaction effect (Table 1). The numbers of coccinellids collected in the yellow sticky cards in spring 2017 did not differ by treatment, and there was no time or interaction effect (Table 2). No coccinellids were collected during the fall of 2017.

The commonly known long-legged flies (Diptera: Dolichopopidae) represent the most abundant predators recorded in both years. Most long-legged flies were collected in yellow sticky traps with at least four species in the genus *Condylostylus*, a group that feeds primarily on soft-bodied arthropods. More long-legged flies were collected in the spring compared with the fall of 2015. Differences among treatments were identified for long-legged flies in spring 2015 ($F_{4,12}$ = 6.50, P = 0.005), with no time or interaction effect. Most treatments showed similar long-legged fly numbers except for the treatment including marigolds mixed with cowpeas which showed higher abundance (Table 1). In fall 2015, the number of long-legged flies differed by treatment ($F_{4,12}$ = 17.83, P<0.0001), with no time or interaction effect. The highest number of long-legged flies was observed in the treatment including Entrust applications followed by the treatment with no pest management (Table 1).

The numbers of long-legged flies differed by treatment in 2017 (spring: $F_{6,18}$ = 13.57; fall: $F_{6,18}$ = 33.01, both P<0.0001), with no time or interaction effect. In spring, yellow sticky traps located in the treatment with marigolds planted alone collected more long-legged flies compared with other treatments. In fall, the highest number was recorded in the treatments including marigolds alone (Table 2).

Naturally occurring parasitoids

Fifty-three morphospecies of parasitoid wasps from 12 families were collected using yellow sticky traps during the 2015 and 2017 experiments. For comparisons across treatments, one dataset including numbers from all morphospecies pooled together as total parasitoid wasps was used for analysis.

There were differences among treatments for the total number of parasitoids collected ($F_{4,12}$ = 51.55, P<0.0001) in spring 2015. Low parasitoid numbers were found in treatments including Entrust applications and no pest management (Table 1). Platygastridae, Encyrtidae, Pteromalidae, Aphelinidae, and Dryinidae were the most abundant parasitoid families collected in spring 2015. Similarly, differences among treatments were identified for numbers of parasitoids in fall 2015 ($F_{4,12}$ = 17.77, P<0.0001). The numbers of parasitoids were higher in the treatment where Entrust was applied followed by treatments including marigolds alone or mixed with cowpeas. Fewer parasitoids were collected in the treatment where cowpeas alone were planted (Table 1). Trichogrammatidae, Platygastridae, and Encyrtidae were most abundant than other parasitoid families in fall 2015.

Parasitoid numbers collected in spring 2017 differed among treatments ($F_{6,18}$ = 18.13, P<0.0001). More parasitoids were collected in treatments including companion plants. The treatment including M-Pede applications showed the lowest number of parasitoids followed by alyssum together with *A. swirskii* release (Table 2). Platygastridae and Mymaridae were the most abundant parasitoid families in spring 2017. The parasitoid numbers in fall 2017 did not differ by treatment; nonetheless, more parasitoids were collected in the fall compared to the spring season (Table 2). Platygastridae and Encyrtidae were the most abundant families in fall 2017.

The predatory mite *Amblyseius swirskii*

There were no significant differences among treatments, time, or interaction for the numbers of *A. swirskii* in spring and fall 2017. In the spring, we did not record any mites 4 WAP (1 week after *A. swirskii* release), but predatory mites were recorded 5 WAP in squash leaves. In the fall, the numbers of *A. swirskii* fluctuated over time. Higher numbers of predatory mites were found on the squash planted next to alyssum or marigold plus *A. swirskii* release 3, 6, and 7 WAP. Fewer predatory mites were recorded on the squash planted together with marigolds alone and the squash without pest management (Figure 2). The predatory mites were not found in companion plants.

Aphids

The most common aphid species recorded were melon aphid and cowpea aphid, *Aphis craccivora* CL Koch. Other species collected included spirea aphid (*Aphis spiraecola* Pach), waterlily aphid (*Rhopalosiphum nymphaeae* L.), apple-grass aphid (*Rhopalosiphum insertum* Walker), rusty plum aphid (*Hysteroneura setariae* Thomas), oil palm aphid (*Schizaphis rotundiventris* Signoret), polygonum aphid (*Capitophorus hippophaes* Walker), and root aphid (*Tetraneura nigriabdominalis* Sasaki).

Most aphids recorded were winged individuals collected using pan traps. Thus, data from pan traps are shown as well as the number of winged aphids found on the companion plants during the in situ counts to identify any aphid reservoirs. In spring 2015, the number of winged aphids collected in pan traps differed by treatment ($F_{4,60} = 4.07$, $P = 0.005$) and over time ($F_{3,60} = 3.32$, $P = 0.02$), with no interaction effect. Aphid numbers were significantly higher in treatments including marigolds alone compared with Entrust and where no pest management was applied (Figure 3A).

Higher numbers of aphids were recorded on the leaves of cowpeas planted alone and on the leaves of marigolds and cowpeas planted together ($F_{2,6} = 11.88$, $P<0.008$), with no time effect, and a significant time*treatment interaction ($F_{10,102} = 7.12$, $P<0.0001$), such that treatment differences were identified 4 and 5 WAP. The numbers of aphids inhabiting the marigolds alone remained low during most of the sampling period (Figure 3B).

In the fall of 2015, the number of winged aphids sampled using pan traps did not differ by treatment, time, and there was no interaction effect (Figure 3A). No aphids were recorded by in situ counts in the companion plants during the fall of 2015.

Low aphid numbers were recorded in 2017, and fewer aphids were recorded in the spring compared with the fall season. In the spring, the number of aphids collected using pan traps differed among treatments ($F_{6,18} = 3.31$, $P = 0.02$), with no time or interaction effect. The highest number of winged aphids was collected in the treatment with marigolds+release of *A. swirskii* in the squash, which was not significantly different to the M-Pede treatment. The treatment including predatory mites alone showed the lowest numbers of aphids (Figure 4A). Hardly any aphids were found inhabiting the companion plants in spring 2017.

In the fall of 2017, the number of winged aphids collected by pan traps did not differ by treatment, time, and there was no interaction effect (Figure 4A). The number of winged and wingless aphids recorded by in situ counts

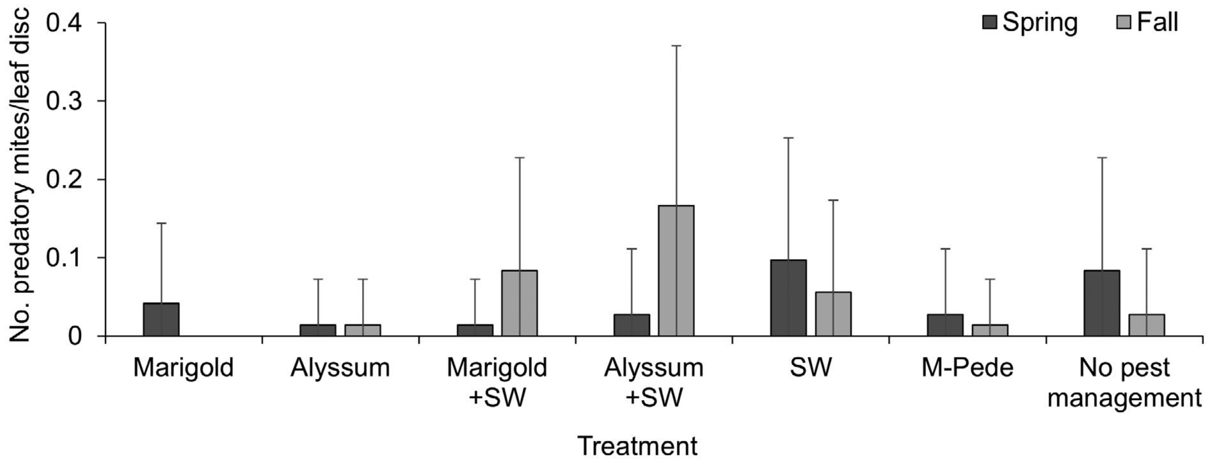

FIGURE 2 Mean (± SE) number of *Amblyseius swirskii* (SW) recorded per leaf disc (4 cm^2) over a 5-week period during spring and fall 2017. Back-transformed data are shown

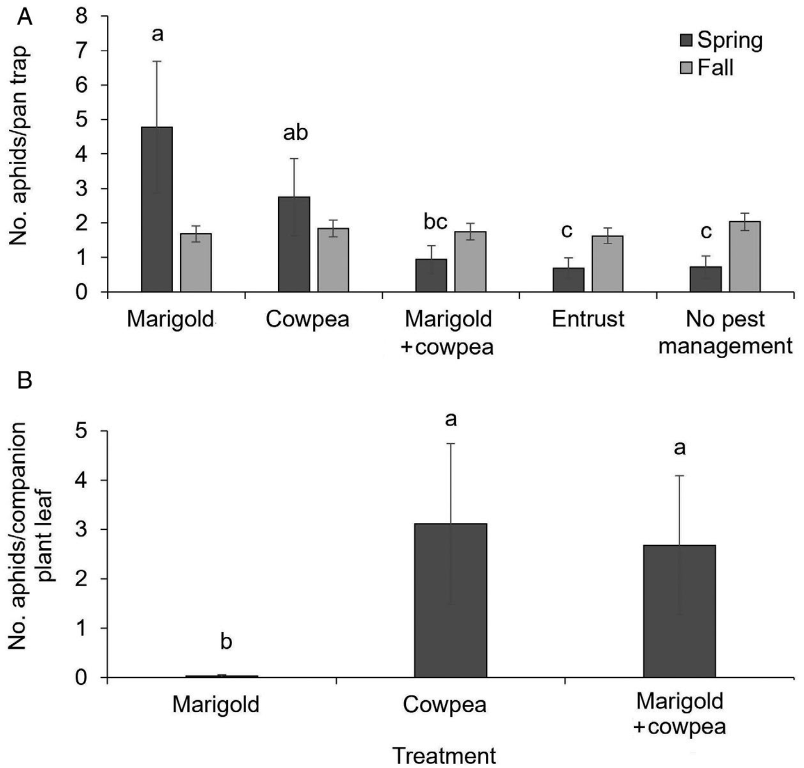

FIGURE 3 Mean (± SE) number of winged aphids sampled over a 5-week period (A) by pan traps in spring and fall, and (B) by in situ counts in the companion plants in spring, in the 2015 experiments. Means within a panel capped with the same letter are not significantly different (Lsmeans: P>0.05)

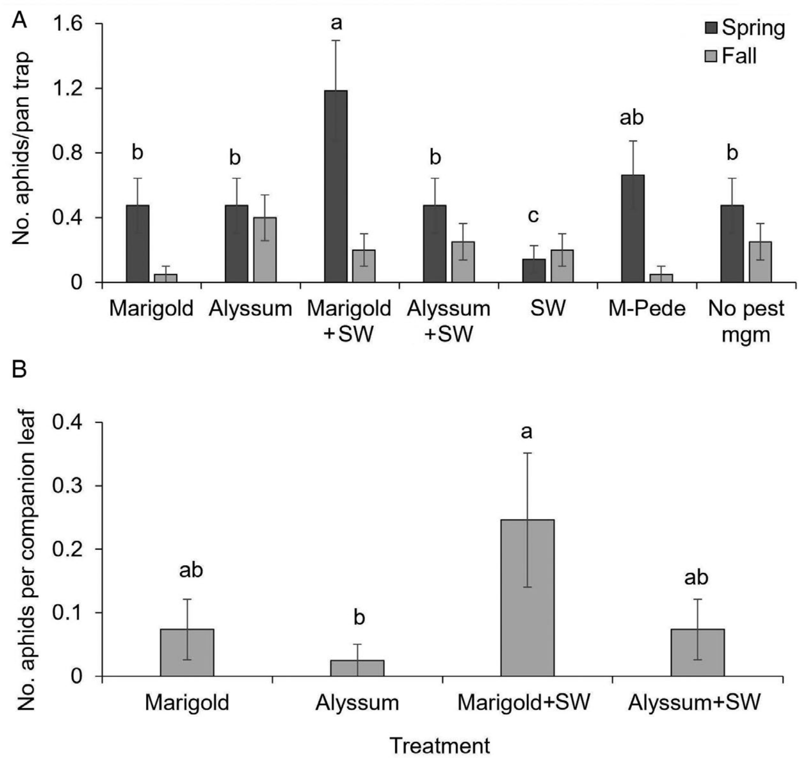

FIGURE 4 Mean (± SE) number of winged aphids sampled over a 5-week period (A) by pan traps in spring and fall, and (B) by in situ counts in the companion plants in fall, in the 2017 experiments. Means within a panel capped with the same letter are not significantly different (Lsmeans: P>0.05). *Amblyseius swirskii* denoted as SW

in the companion plants differed by treatment (winged: $F_{3,18}$ = 346.48, P<0.0001; wingless: $F_{3,18}$ = 6.64, P = 0.001), with no time or interaction effect. Across treatments, more aphids were found in the marigolds compared with the alyssums (Figure 4B).

Aphid-transmitted viruses

Aphid-transmitted viruses were detected at low incidence in fall 2015 and 2017. In 2015, viral infection with PRSV was present in samples from all treatments, ZYMV was detected in treatments where companion plants were planted alone or no pest management was implemented, WMV was only detected in one sample from squash planted next to cowpeas alone, and CMV was not detected in any of the processed leaf samples. Only one squash sample from the treatment including cowpeas alone showed viral infection with all three viruses (Table 3). In 2017, ELISA assays for the *Potyvirus* group showed up to three samples infected in the treatment where *A. swirskii* was released and no companion plant was used (Table 4).

Whiteflies

Marigold, cowpea, and alyssum were not used by whiteflies as a host plant during the study as no oviposition or immature stages were found on these companion plants. However, varying infestation levels of sweetpotato whiteflies were observed in the squash crop during 2015 and 2017.

A lower whitefly infestation was observed during the spring of 2015 compared with the fall. Hardly any whitefly adults were collected on yellow sticky traps across treatments (mean ± SE = 0.09 ± 0.07 whiteflies per trap) during the spring of 2015 (data not included in figures). In fall 2015, the number of whiteflies collected on yellow sticky traps differed by treatment ($F_{4,12}$ = 2.61, P = 0.08), with no time or interaction effect (Figure 5A). High numbers of whiteflies (>20 whiteflies per trap) infested the squash during most of the sampling period. The highest number of whiteflies was collected in the treatment with Entrust followed by the treatments with cowpeas alone and no pest management, which were not significantly different from each other (Figure 5A).

Following a similar pattern to those of whitefly adults, low numbers of immature whiteflies (0.55 ± 0.36 immature whiteflies per disc) were found in the squash in spring 2015. In fall 2015, more whitefly immatures were recorded in the squash treated with Entrust (15.5 ± 3.4 immatures per disc) and the squash planted next to the cowpeas alone (14.5 ± 3.3 immatures per disc) (data not shown in figures). Yet, there were no significant differences among treatments, time, or interaction effect for whitefly immatures recorded in the spring and fall of 2015.

A different whitefly population pattern was observed in 2017 with higher numbers of adult and immature whiteflies during the spring compared with the fall season. In spring 2017, the numbers of adult whiteflies collected differed by treatment ($F_{6,18}$ = 2.80, P = 0.04), with a time ($F_{4,12}$ = 115.82, P<0.0001) and interaction effect ($F_{24,72}$ = 1.89, P = 0.02), such that significant treatment differences were found 7 WAP. The lowest number of whitefly adults was collected in the treatment where *A. swirskii* was released with no companion plants, whereas the remaining treatments showed similar whitefly abundances. In fall 2017, there were no significant differences among treatments, and no time or interaction effects for the abundance of whitefly adults (Figure 5B).

The numbers of whitefly immatures recorded did not differ by treatment or time, but there was a significant time*treatment interaction in spring ($F_{14,72}$ = 18.2, P<0.0001) and fall 2017 ($F_{21,90}$ = 1.86, P = 0.02). An average of 1.95 ± 0.70 and 0.65 ± 0.40 immatures per disc were recorded in spring and fall, respectively (data not shown in figures). In spring, low numbers of whitefly immatures were recorded in the squash overall treatments during the first 3 weeks of sampling (0.21 ± 0.14 immatures per disc). Immature numbers increased 6 and 7 WAP (4.94 ± 2.49 and 11.94 ± 5.72 immatures per disc, respectively) with the highest numbers in squash with alyssum alone as companion plant, and alyssum together with *A. swirskii* release in the squash (data not shown in figures). In fall 2017, slightly more immature whiteflies were recorded in the squash planted next to alyssum alone and alyssum+*A. swirskii* release (0.21 ± 0.13 and 0.23 ± 0.14 immatures per disc, respectively), whereas fewer immatures were found in the squash planted next to marigolds alone and squash treated with M-Pede (0.12 ± 0.08 and 0.12 ± 0.08 immatures per disc) (data not shown in figures).

TABLE 3 Percentage of samples (numbers in parenthesis) showing viral infection with one of four plant viruses (*Papaya ringspot virus*, PRSV; *Zucchini yellow mosaic virus*, ZYMV; *Watermelon mosaic virus*, WMV; or *Cucurbit leaf crumple virus*, CuLCrV) during fall 2015

Treatment	PRSV (n/80[a])	ZYMV (n/80[a])	WMV (n/80[a])	CuLCrV (n/40[b])
Marigold	1.25 (1)	1.25 (1)	0	17.5 (7)
Cowpea	1.25 (1)	1.25 (1)	1.25 (1)	20.0 (8)
Marigold+cowpea	3.75 (3)	0	0	20.0 (8)
Entrust (spinosad)	2.50 (2)	0	0	12.5 (5)
No pest management	1.25 (1)	1.25 (1)	0	12.5 (5)

[a]80 samples (four per plot) used for ELISA assays.
[b]40 samples (two per plot) used for PCR.

TABLE 4 Percentage of samples (numbers in parenthesis) showing viral infection with any plant virus from the genus *Potyvirus* or *Cucurbit leaf crumple virus* (CuLCrV) during fall 2017

Treatment	*Potyvirus* group (n/56[a])	CuLCrV (n/56[a])
Marigold	1.79 (1)	8.93 (5)
Alyssum	0	10.71 (6)
Marigold + *A. swirskii*	1.79 (1)	7.14 (4)
Alyssum + *A. swirskii*	3.57 (2)	12.50 (7)
A. swirskii only	5.36 (3)	8.93 (5)
M-Pede (soap concentrate)	1.79 (1)	10.71 (6)
No pest management	1.79 (1)	8.93 (5)

Potyvirus group: PRSV, WMV, ZYMV, and CMV.
[a]56 samples (two per plot) used for ELISA assays and PCR.

Whitefly-transmitted/induced diseases

No significant differences were found among treatments for the averaged SSL index in spring and fall 2015 (Figure 6). Low

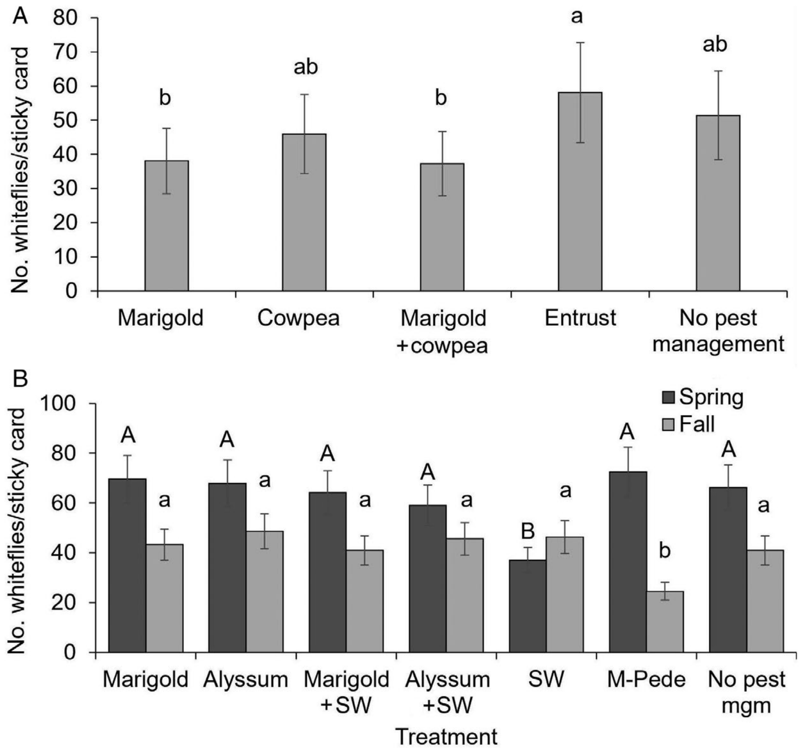

FIGURE 5 Mean (± SE) number of adult whiteflies sampled by yellow sticky traps over a 5-week period in (A) fall 2015 and (B) spring and fall 2017. Means within a panel and within a season capped with the same letter are not significantly different (Lsmeans: P>0.05). *Amblyseius swirskii* denoted as SW

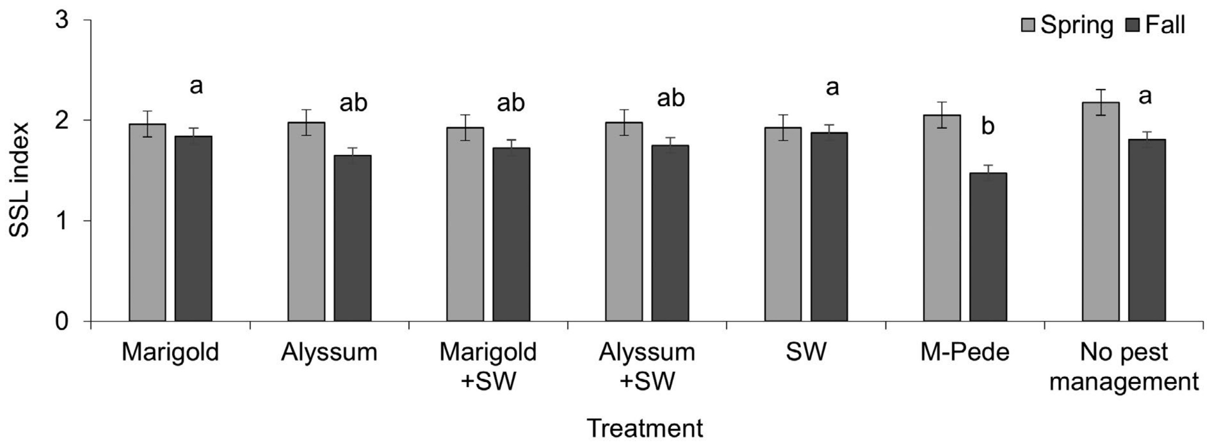

FIGURE 6 Mean (± SE) squash silverleaf (SSL) disorder index rated in spring and fall 2017. Back-transformed data are shown. Means capped with the same letter are not significantly different (Lsmeans: P>0.05). *Amblyseius swirskii* denoted as SW

SSL incidence (mean index ± SE = 1.34 ± 0.08) was observed during the spring with plants showing secondary veins silvered. Nonetheless, high SSL ratings were observed during the fall with averaged rating 3 ± 0.1 in treatments where cowpeas were planted alone. This means most plants showed extended silvering between primary and secondary veins.

There were no significant differences among treatments for the averaged SSL index in spring 2017 (Figure 6). In fall 2017, differences were observed among treatments ($F_{6,30}$ = 3.77, P = 0.006). All ratings were below 2 meaning that most plants showed various leaves with veins pale and appearing 'netted'; however, the treatment where M-Pede was sprayed showed an average index closer to 1 where plants showed no netted appearance and only secondary veins silvered (Figure 6).

Squash plants screened for *CuLCrV* showed viral infection in 83% (33 out of 40) of the samples tested in fall 2015. The virus was detected at similar incidence across treatments (Table 3). Likewise, CuLCrV was detected in 63% (38 out of 56) of the samples assayed in fall 2017. *Cucurbit leaf crumple virus* incidence appeared not to be influenced by the presence of the companion plants or the release of the predatory mites (Table 4).

Yield

No significant differences among treatments were found for the total fruit injured by pickleworms (marketable and unmarketable yield) in 2015 experiments. The amount of unmarketable fruit was almost equal to the amount of marketable fruit in both the spring and fall season. Additionally, the marketable yield was approximately 40% less in the fall and the total fruit injured by pickleworms increased considerably compared to the spring of 2015 (Figure 7A,B).

There were differences among treatments in the marketable ($F_{6,24}$ = 5.04, P = 0.001) and unmarketable yield ($F_{6,22}$ = 3.10, P = 0.02) in spring 2017. Higher yields were obtained from the squash treated with M-Pede followed by the squash planted next to the marigolds+release of *A. swirskii*.

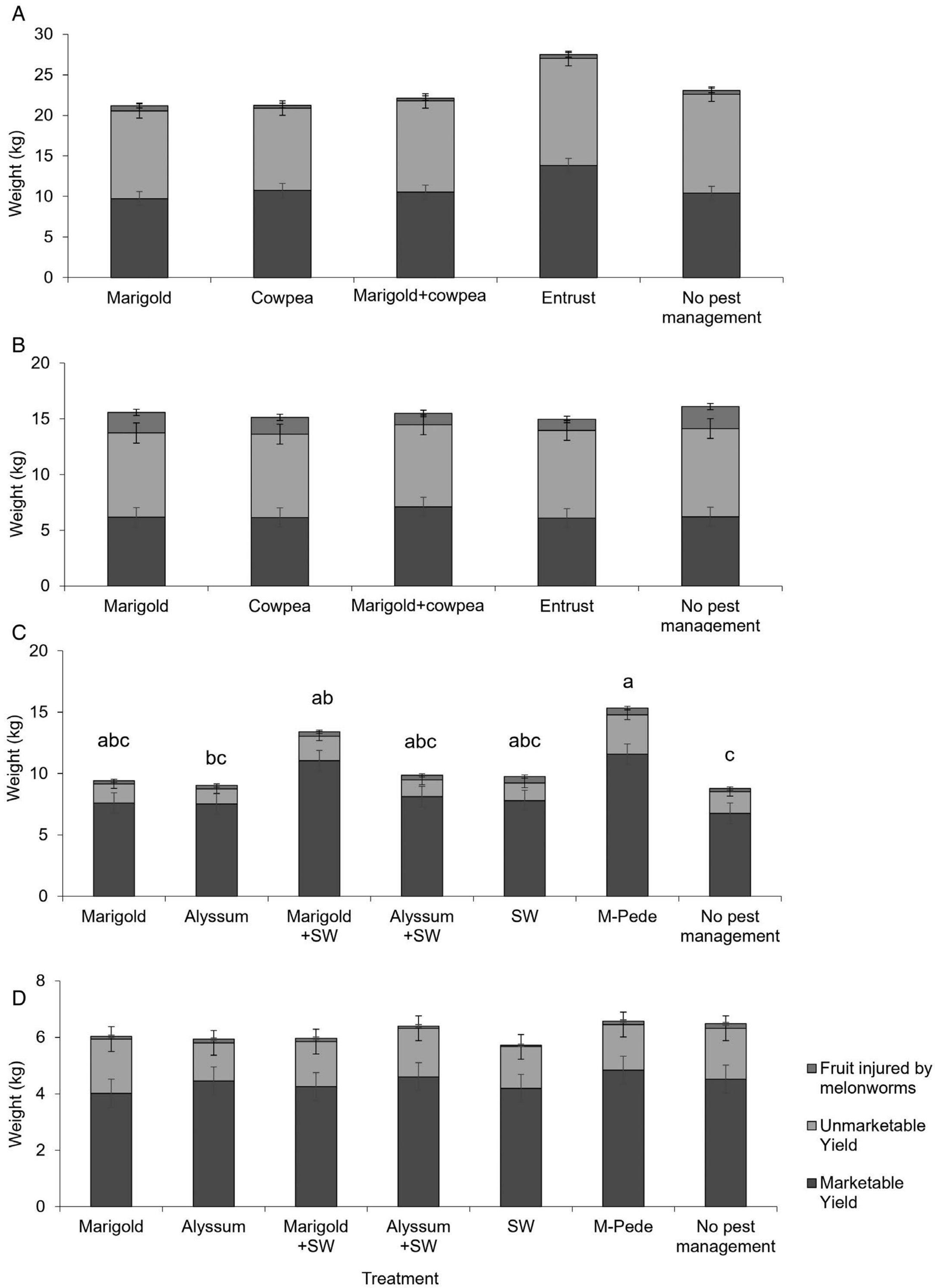

FIGURE 7 Mean (± SE) total (kg) marketable yield, unmarketable yield, and fruit injured by pickleworms harvested per treatment in (A) spring 2015, (B) fall 2015, (C) spring 2017, and (D) fall 2017. Back-transformed data are shown. Combined totals within a panel capped with the same letter are not significantly different (Lsmeans: P>0.05). *Amblyseius swirskii* denoted as SW

The lowest yield was recorded in the control where no pest management was implemented (Figure 7C,D). There were no significant differences among treatments for fruit injured by pickleworms (marketable and unmarketable yield) in fall 2017. Nonetheless, the same tendency observed in 2015 was found in 2017 where there was an approximately 50% reduction in the marketable yield recorded in fall compared with the spring season (Figure 7C,D).

DISCUSSION

Our findings indicate that all three companion plants evaluated caused the build-up of natural enemies around the organic squash plants. Eighty-two morphospecies of beneficial arthropods were identified during the experiments; however, only a few beneficial arthropods moved from the companion plants towards the squash plants. The suppressive effects of using marigolds and sweet alyssum with predatory mites were comparable to the use of *A. swirskii* alone with the additional advantage of predators and parasitoids attracted to the companion plants, which increases the potential to suppress other squash pests such as aphids and thrips.

Natural enemies

Cowpeas were attractive to adult and immature stages of coccinellids that were observed feeding on aphids inhabiting the plants, as well as one species in the genus *Delphastus*. This is a group that feeds only on whitefly species; however, there was no evidence that *Delphastus* sp. had any effect on whitefly populations in the squash. Predatory insects and parasitoid wasps were also attracted by the cowpeas. Similar results were reported by Tanga & Niba (2019), who evaluated the attractiveness of mulitple cowpea cultivars to beneficial arthropods. Authors concluded that all cowpea cultivars were highly attractive to natural enemies particularly to predators, parasitoids, and pollinators in the order Hymenoptera; however, cowpea cultivars were also highly susceptible to pest infestations.

Marigolds were also attractive to several parasitoids and predatory species during this study. Predators such as *Orius* sp. were drawn to the marigolds in search of food and shelter. We believe that *Orius* sp. was able to use marigolds as a host for reproduction and development because thrips were always present in the marigold flowers and may have served as the primary food source for *Orius* sp. However, despite the high population of *Orius* sp., there was no evidence that they had any effect on pest species present in organic squash.

Multiple studies have assessed sweet alyssum as an insectary plant intercropped with cabbage, lettuce, and pepper, among other vegetable crops, for management of aphids by the attraction of predatory flies and parasitoid wasps (e.g., braconid wasps) (Bugg et al., 2008; Hogg, 2011; Brennan, 2016, 2013). In our study, sweet alyssum was attractive to whitefly parasitoids, including aphelinid and platygastrid wasps. Sweet alyssum was also attractive to predators such as *Orius* sp., and in many instances, syrphid flies were observed visiting alyssum flowers, but hardly any syrphids were collected by the sampling methods implemented. In contrast, high numbers of long-legged flies were collected around alyssum planted alone or together with release of *A. swirskii*. At least four species from the genus *Condylostylus* were collected during the studies. These flies were observed actively feeding on aphids during the squash season and represented the dominant predator in the study. In Florida at least 20 genera of *Condylostylus* are known to exist (GJ Steck, pers. comm.) and large populations commonly established throughout the year (Cicero et al., 2017). Generally, dolichopopid flies have predacious adults and have been reported to feed on fungus gnats, leaf-miner flies, aphids, leafhoppers, thrips, whiteflies, and mites (Cicero et al., 2017).

Populations of *A. swirskii* have been established in other cucurbits such as cucumbers for control of thrips (Kakkar et al., 2016), but few studies have reported the establishment of reproductive populations of this predatory mite in squash crops. This study represents one of the few attempts to release and establish *A. swirskii* in squash and open-field production.

The number of *A. swirskii* mites recovered was lower than expected. Environmental conditions may have influenced the numbers of *A. swirskii* collected. For example, important rain events in spring 2017 (more than four rainy days within the week, 10–35 mm per day) following the release of the predatory mites could have limited their establishment. Likewise, temperatures <20 °C and close to 10 °C by the end of the season may have contributed to the reductions in predatory mite numbers observed in fall 2017. The presence of glandular trichomes in the squash leaves, especially in younger leaves, may have prevented the establishment of larger populations of this predatory mite. *Amblyseius swirskii* was hardly observed in younger leaves where these trichomes are most abundant. Calvo et al. (2015) and Xiao et al. (2012) reported that *A. swirskii* are more likely to establish in crops with glabrous leaves such as peppers or cucumbers. Low numbers of *A. swirskii* may also be explained by their high dispersal capacity. *Amblyseius swirskii* are highly mobile and can move from plant to plant using the connections between the leaves and plastic mulch as bridges (Lopez et al., 2017). Moreover, they can be airborne, especially gravid females in search of prey or alternative food sources.

Aphids

Cowpeas were highly attractive to cowpea aphids during the 2015 experiments. High numbers of aphids were recorded mostly during the spring of 2015, and fungus proliferation occurred due to continuous secretion of honeydew on top of the leaves and plastic mulch. Aphids were present in the squash in high numbers by the beginning of the sampling period suggesting that they colonized the plants at an early developmental stage when plants had fewer than five true leaves and were less than 25 cm high. In Florida aphid females reproduce year-round without mating, thus, populations are always ready to colonize newly planted crops. Mixing cowpeas with marigolds continued to harbor large numbers of aphids. Cowpea aphids are not commonly found in cucurbits, but high numbers of aphids on the cowpeas caused a spill-over effect on the squash planted near cowpeas alone and cowpeas mixed with marigolds.

Previous studies have demonstrated that cowpeas alone or intercropped with marigolds are susceptible to severe infestations by *A. craccivora* and other insect pests (Tanga & Niba, 2019). Martinez et al. (2020) also demonstrated the effect of cowpeas on insect pests when used as cover crop before squash planting. Authors showed that squash grown after cowpea cover crops suffered significantly higher pest damage compared with squash grown after other evaluated cover crops. Despite the important role that cowpeas played as refugia for beneficial arthropods in our study, it also became a reservoir of aphids that were dispersing towards the neighboring squash and put the crop at risk. Therefore, cowpea is not recommended as a companion plant or trap crop within squash cropping systems.

Despite the diversity of aphid species identified in 2017, most of them were not pests of cucurbits except for melon aphid. Aphid species such as root aphid, rusty plum aphid, and cowpea aphid are commonly found in Florida feeding on grasses, weeds, or other crops (e.g., cowpeas) and are non-colonizing aphids in the squash (S Halbert, pers. comm.). However, a few of these aphid species are reported as potential vectors of plant viruses and they potentially transmit viruses to the squash. Therefore, it is important to monitor non-colonizing aphid species as well as winged and wingless stages, especially when other plant species are grown in proximity.

In 2017, marigolds were observed harboring significantly larger populations of aphids compared to sweet alyssum. Yet, the spill-over of aphids from the cowpeas to the squash observed in 2015 was not observed from the marigolds to the squash in 2017. This could be related to differences in the infestation levels between 2015 and 2017. In 2017, the level of aphid infestation was low (<5 aphids per companion plant leaf) whereas in 2015, up to 18 aphids were counted on a single companion plant leaf damaging the companion plants very rapidly and forcing the aphids to search for neighboring plant hosts including the squash.

Marigolds seemed to play a role as a trap crop for aphids during the 2017 experiments. Trap crops are used to attract, repel, or intercept insects or the pathogens they vector to reduce pest numbers and disease incidence in the crop (Badenes-Perez, 2019). The potential of marigolds as a trap crop for the management of aphids has been evaluated by Jankowska et al. (2009). Authors reported that the total numbers of cabbage aphids, *Brevicoryne brassicae* L., present in cabbage intercropped with marigolds was 2–7× lower compared with cabbage monoculture. The potential of marigolds as a trap crop for aphid species in squash could have important benefits for disease management programs considering the mode of virus transmission involved in aphid-virus interactions. Aphids can transmit multiple plant viruses at the same time within short periods of time because the virus is attached to the tip of their stylets. Viral particles are easily inoculated into the tissues of healthy plants when aphids probe new hosts as a method to examine the quality of the plant (Mauck et al., 2012). Due to aphids' probing behavior, we believe that marigolds used as traps crops potentially reduce squash viral infection as the aphids' probe and feed on the non-crop plants and deplete their viral inoculum (Zavaleta-Mejía & Gomez, 1995; Mauck et al., 2012).

Whiteflies

Whitefly populations colonized the squash at the early stages of the crop, similar to aphids. High numbers of whitefly immatures feeding on the squash in fall 2015 worsened the SSL symptoms resulting in multiple plants showing extensive silvering. Contrary to the SSL disorder caused by immature feeding, CuLCrV is transmitted by whitefly adults causing plant stunting and severe leaf and fruit malformations. *Cucurbit leaf crumble virus* together with high SSL incidence appeared to have a detrimental effect on plant fitness. The combination of whitefly-transmitted diseases with high insect pest infestations seemed to cause a ca. 40% reduction in marketable yield during the fall compared with the spring of 2015 when no viral diseases were observed, and low aphid and whitefly infestation levels were recorded.

Entrust is an organically approved pesticide commonly used by growers as one of the primary tools for the management of soft-bodied insect pests in organic squash production. However, it was not effective suppressing aphid and whitefly populations during the 2015 experiments. Thus, M-Pede was used as an alternative pesticide for the control treatment in the 2017 experiments. Fewer whiteflies were recorded in the organic squash treated with M-Pede during the fall 2017 experiments, which was reflected in the SSL symptoms with more squash showing mild levels of silvering. Nonetheless, only the squash treated with the organic labelled insecticide M-Pede show significantly lower levels of SSL. Razze et al. (2016b) also demonstrated the effectiveness of M-Pede against sweetpotato whiteflies.

During the squash season in North-Central Florida, it is common to have higher whitefly pressure during the fall than during the spring. This tendency was observed in 2015 experiments; however, in 2017 high whitefly pressure was observed during the spring with up to 80 adult whiteflies per trap. Traps from fall continued to show high numbers (ca. 60 per trap) at the beginning of the season, but numbers slowly declined during the following weeks. The decline of whitefly populations during fall 2017 may be related to lower temperatures and the presence of *A. swirskii*.

In 2017 experiments, the predatory mites appeared to have provided biocontrol services resulting in the reduction of whitefly numbers. Immature whiteflies in the same treatment did not show the lowest values but showed low abundances compared to other treatments. *Amblyseius swirskii* feeds on whitefly eggs and on first and second instars (Soleymani et al., 2016). Substantial reductions in eggs and immature stages due to predator feeding appeared to explain the low numbers of whiteflies recorded in selected plots in spring 2017. The same reduction in whitefly immatures was observed in fall 2017 when *A. swirskii* was released without the presence of companion plants. Whitefly immatures were low the week when the predatory mites were released (4 WAP), and low numbers continued to occur for the rest of the experiment, potentially indicating that *A. swirskii* provided some regulation of the whitefly population.

The use of *A. swirskii* without companion plants showed lower numbers of whitefly adults, but this reduction in whiteflies was not reflected in higher marketable yields. *Amblyseius swirskii* can be used as a management tactic in organic squash against whiteflies and thrips (Kakkar et al., 2016) but should not be used as the only management tool as the crop could be negatively affected by aphids. The predatory mites can feed on pests such as whiteflies and thrips but do not feed on aphids. Choosing the most appropriate companion plant (between marigolds and alyssum for releases with *A. swirskii*) should be based on the pests that pose a major threat in the cropping system. For example, if aphid pressure together with a high level of aphid-transmitted viruses are the main risk to the squash crop, marigolds would be an appropriate companion plant together with *A. swirskii*. The predatory mites could suppress whitefly and/or thrips populations whereas marigolds are used as a trap crop for aphids that mitigate or delay the spread of aphid-transmitted viruses into the squash. In contrast, if whiteflies and whitefly-transmitted viruses represent the major threat to the squash crop, alyssum may be an appropriate companion plant together with *A. swirskii* releases. Sweet alyssum is especially attractive to aphid predators and parasitoids that could maintain low-to-moderate aphid populations in check while *A. swirskii* suppress whiteflies in the squash.

Lastly, recent studies have found significant negative relationships between plant diversification and pest injury in vegetable agroecosystems, as well as the increase in beneficial arthropods when trap crops and insectary plants are used together as a push–pull tactic (Shrestha et al., 2019; Juventia et al., 2021). Based on these findings, future research could include the evaluation of a multi-tactic approach with additional levels of habitat complexity where marigolds are established as trap crop exposed to sporadic insecticide applications, sweet alyssum are grown as insectary plant to attract beneficial arthropods, and *A. swirskii* is released in the squash as a complementary tactic.

CONCLUSION

This study aimed to determine whether a combined approach including conservation (companion plants) and augmentative (*A. swirskii* release) biocontrol techniques can increase the biocontrol services of key pests in organic squash. All companion plants used in this study increased natural enemies within their respective treatments, but only African marigolds and sweet alyssum ultimately increased biocontrol activities. The combined effects of *A. swirskii* and naturally-occurring predators, such as *Orius* sp. and long-legged flies, attracted to the companion plants appeared to have caused reductions in whitefly infestation levels in selected plots. Therefore, we believe that both marigolds and sweet alyssum showed good performance as companion plants within the squash cropping system. Whereas marigolds showed potential as a trap crop for aphids, hardly any key pest was recorded in the alyssum, making sweet alyssum an optimal insectary plant for the squash. The combined techniques used in this study can be adopted by organic and conventional squash growers and other cucurbit producers in Florida and the rest of the southern USA (e.g., Georgia) that are challenged by similar insect pests and disease pressure. Enhancing biocontrol services will reduce the number of insecticide applications, as well as pollinators' and farmers' exposure to toxic chemicals.

ACKNOWLEDGMENTS

We thank Salvador Gezan for his assistance with the statistical analysis. This work was supported by Specialty Crop Block Grant Program, Florida Department of Agriculture and Consumer Services (FDACS) under grant #022921 and Graduate Student Grant in Sustainable Agriculture, Southern Sustainable Agriculture Research and Education (SSARE) under grant #RD309-129/S000843.

CONFLICT OF INTEREST

There is no conflict of interest that could be perceived as prejudicing the impartiality of this research.

AUTHOR CONTRIBUTION

Lorena Lopez: Data curation (lead); Formal analysis (lead); Funding acquisition (equal); Investigation (lead); Methodology (lead); Writing – original draft (lead); Writing – review & editing (equal). Oscar Emanuel Liburd: Funding acquisition (equal); Methodology (equal); Project administration (lead); Supervision (lead); Writing – review & editing (equal).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Lorena Lopez https://orcid.org/0000-0003-3123-3715
Oscar E. Liburd https://orcid.org/0000-0001-8827-5823

REFERENCES

Akad F, Webb S, Nyoike TW, Liburd OE, Turechek W et al. (2008) Detection of cucurbit leaf crumple virus in Florida cucurbits. *Plant Disease 92*: 648.

Badenes-Perez F (2019) Trap crops and insectary plants in the order Brassicales. *Annals of the Entomological Society of America 112*: 318–329.

Brennan EB (2016) Agronomy of strip intercropping broccoli with alyssum for biological control of aphids. *Biological Control 97*: 109–119.

Brennan EB (2013) Agronomic aspects of strip intercropping lettuce with alyssum for biological control of aphids. *Biological Control 65*: 302–311.

Bugg RL, Colfer RG, Chaney WE, Smith HA & Cannon J (2008) Flower flies (Syrphidae) and other biological control agents for aphids in vegetable crops. *University of California Division of Agriculture and Natural Resources 82*: 1–25.

Buitenhuis R, Murphy G, Shipp L & Scott-Dupree C (2015) *Amblyseius swirskii* in greenhouse production systems: a floricultural perspective. *Experimental and Applied Acarology 65*: 451–464.

Calvo JF, Knapp M, van Houten YM, Hoogerbrugge H & Belda JE (2015) *Amblyseius swirskii*: what made this predatory mite such a successful biocontrol agent? *Experimental and Applied Acarology 65*: 419–433.

Calvo FJ, Bolckmans K & Belda JE (2011) Control of *Bemisia tabaci* and *Frankliniella occidentalis* in cucumber by *Amblyseius swirskii*. *BioControl 56*: 185–192.

Cicero J, Adair MM, Adair RC, Hunter WB, Avery PB & Mizell RF (2017) Predatory behavior of long-legged flies (Diptera: Dolichopodidae) and their potential negative effects on the parasitoid biological control agent of the Asian citrus psyllid (Hemiptera: Liviidae). *Florida Entomologist 100*: 485–487.

El-Gindi AY, Osman HA, Youseef MMA, Ameen HH & Lashein AM (2005) Evaluation of the nematicidal effects of some organic amendments, biofertilizers and intercropped marigold, *Tagetes erecta* plant on the root-knot nematode, *Meloidogyne incognita*-infected cowpea plants. *Bulletin of the National Research Centre 30*: 307–315.

Farkas P, Bagi N, Szabó Á, Ladányi M, Kis K et al. (2016) Biological control of thrips pests (Thysanoptera: Thripidae) in a commercial greenhouse in Hungary. *Polish Journal of Entomology 85*: 437–451.

Gillespie M, Wratten S, Sedcole R & Colfer R (2011) Manipulating floral resources dispersion for hoverflies (Diptera: Syrphidae) in a California lettuce agro-ecosystem. *Biological Control 59*: 215–220.

Gontijo LM, Beers EH & Snyder WE (2013) Flowers promote aphid suppression in apple orchards. *Biological Control 66*: 8–15.

Hagen C, Rojas MR, Sudarshana MR, Xoconostle-Cazares B, Natwick ET et al. (2008) Biology and molecular characterization of cucurbit leaf crumple virus an emergent cucurbit-infecting *Begomovirus* in the Imperial Valley of California. *Plant Disease 92*: 781–793.

Hogg BN, Nelson EH, Mills NJ & Daane KM (2011) Floral resources enhance aphid suppression by a hoverfly. *Entomologia Experimentalis et Applicata 141*: 138–144.

Jankowska B, Jedrszczyk E & Poniedzialek M (2012) Effect of intercropping carrot (*Daucus carota* L.) with French marigold (*Tagetes patula nana* L.) and pot marigold (*Calendula officinalis* L.) on the occurrence of some pests and quality of carrot yield. *Acta Agrobotanica 65*: 133–138.

Jankowska B, Poniedziałek M & Jędrszczyk E (2009) Effect of intercropping white cabbage with French Marigold (*Tagetes patula nana* L.) and Pot Marigold (*Calendula officinalis* L.) on the colonization of plants by pest insects. *Folia Horticulturae 21*: 95–103.

Juventia SD, Rossing WAH, Ditzler L & van Apeldoorn DF (2021) Spatial and genetic crop diversity support ecosystem service delivery: a case of yield and biocontrol in Dutch organic cabbage production. *Field Crops Research 261*: 108015.

Kakkar G, Kumar V, Seal DR, Liburd OE & Stansly PA (2016) Predation by *Neoseiulus cucumeris* and *Amblyseius swirskii* on *Thrips palmi* and *Frankliniella schultzei* on cucumber. *Biological Control 92*: 85–91.

Kheirodin A, Simmons AM, Legaspi JC, Grabarczyk EE, Toews MD et al. (2020) Can generalist predators control *Bemisia tabaci*? *Insects 11*: 823.

Koppert Biological Systems (2022) *Swirskii-Mite*. https://www.koppertus.com/swirski-mite/ accessed on 10 January 2022.

Koptur S, Jones IM & Pena JE (2018) The influence of host plant extrafloral nectaries on multitrophic interactions: an experimental investigation. *PLoS ONE 13*: e0202836.

Kutuk H, Karacaoglu M, Tufekli M & Villanueva RT (2016) Failure of biological control of *Frankliniella occidentalis* on protected eggplants using *Amblyseius swirskii* in the Mediterranean region of turkey. *Turkish Journal of Agriculture and Forestry 40*: 13–17.

Letourneau DK (1990) Mechanisms of predator accumulation in a mixed crop system. *Ecological Entomology 15*: 63–69.

Lopez L, Smith HA, Hoy MA & Cave RD (2017) Dispersal of *Amblyseius swirskii* (Acari: Phytoseiidae) on high-tunnel bell peppers in presence or absence of *Polyphagotarsonemus latus* (Acari: Tarsonemidae). *Journal of Insect Science 17*: 6.

Martinez L, Soti P, Kaur J, Racelis A & Kariyat RR (2020) Impact of cover crops on insect community dynamics in organic farming. *Agriculture 10*: 209.

Mauck K, Bosque-Pérez NA, Eigenbrode SD, De Moraes CM & Mescher MC (2012) Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. *Functional Ecology 26*: 1162–1175.

McAvoy G (2016) Cucurbit yellow stunting disorder virus cases flaring in south Florida. Growing Produce. Available at: http://www.growingproduce.com/crop-protection/disease-control/cucurbit-yellow-stunting-disorder-virus-cases-flaring-in-south-florida/(accessedon 10 December 2020).

Messelink GJ, van Maanen R, van Holstein-Saj R, Sabelis MW & Janssen A (2010) Pest species diversity enhances control of spider mites and whiteflies by a generalist phytoseiid predator. *BioControl 55*: 387–398.

Mossler MA & Nesheim ON (2011) *Florida crop/Pest management profile: Squash1*. University of Florida, Agronomy Department, UF/IFAS Extension, Gainesville, FL, USA. Available at: https://www.growables.org/informationVeg/documents/SquashCropPestManagement.pdf (accessed on 5 January 2021).

Nomikou M, Janssen A, Schraag R & Sabelis MW (2002) Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. *Experimental and Applied Acarology 27*: 57–68.

Nyoike TW & Liburd OE (2010) Effect of living (buckwheat) and UV reflective mulches with and without imidacloprid on whiteflies, aphids and marketable yields of zucchini squash. *International Journal of Pest Management 56*: 31–39.

Nyoike TW, Liburd OE & Webb SE (2008) Suppression of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae) and incidence of cucurbit leaf crumple virus, a whitefly-transmitted virus of zucchini squash new to Florida, with mulches and imidacloprid. *Florida Entomologist 91*: 460–465.

Razze JM, Liburd OE & Webb SE (2016a) Intercropping buckwheat with squash to reduce insect pests and disease incidence and increase yield. *Agroecology and Sustainable Food Systems 40*: 863–891.

Razze JM, Liburd OE, Nuessly GS & Samuel-Foo M (2016b) Evaluation of bioinsecticides for management of *Bemisia tabaci* (Hemiptera:

Aleyrodidae) and the effect on the whitefly predator *Delphastus catalinae* (Coleoptera: Coccinellidae) in organic squash. *Journal of Economic Entomology 109*: 1766–1771.

Shrestha B, Finke DL & Pinero JC (2019) The 'botanical triad': the presence of insectary plants enhances natural enemy abundance on trap crop plants in an organic cabbage agro-ecosystem. *Insects 10*: 181.

Silveira LC, Berti E, Pierre LS, Salles F & Cassa JN (2009) Marigold (*Tagetes erecta* L.) as ann attractive crop to natural enemies in onion fields. *Scientia Agricola 66*: 780–787.

Skirvin DJ, Kravar-Garde L, Reynolds K, Wright C & Mead A (2011) The effect of within-crop habitat manipulations on the conservation biological control of aphids in field-grown lettuce. *Bulletin of Entomological Research 101*: 623–631.

Soleymani S, Hakimitabar M & Seiedy M (2016) Prey preference of predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae) on *Tetranychus urticae* (Acari: Tetranychidae) and *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Biocontrol Science and Technology 26*: 562–569.

Stansly PA, Castillo JA, Tansey JA & Kostyk BC (2018) Management of insect and mite pests with predaceous mites in open-field vegetable crops. *Israel Journal of Entomology 48*: 83–111.

Stansly PA & Natwick ET (2010) Integrated systems for managing *Bemisia tabaci* in protected and open field agriculture. *Bemisia: Bionomics and Management of a Global Pest* (ed. by PA Stansly & SE Naranjo), pp. 467–497. Springer, Dordrecht, The Netherlands.

Stansly PA & Castillo J (2009) Control of broad mite, *Polyphagotarsonemus latus* and the whitefly *Bemisia tabaci* in open field pepper and eggplant with predaceous mites. *IOBC/WPRS Bulletin 49*: 145–152.

Tanga M & Niba AS (2019) Effects of cowpea (*Vigna unguiculata* (L) Walp. farming systems on arthropod community structure in a grassland agro-ecosystem, South Africa. *African Journal of Food, Agriculture, Nutrition and Development 19*: 14873–14888.

VSCNews (2020) Whiteflies impacting Alabama vegetables. *Vegetable and Specialty Crop News*. Available at: https://vscnews.com/alabama-whiteflies-vegetables/ (accessed on 21 July 2021).

Wang KH (2012) Cover crops as insectary plants to enhance above and below ground beneficial organisms. *The Food Provider*. Available at: https://kohalacenter.org/docs/resources/hpsi/InsectaryCoverCrops.pdf (accessed on 18 December 2020).

Whitfield AE, Falk BW & Rotenberg D (2015) Insect vector-mediated transmission of plant viruses. *Virology 479/480*: 278–289.

Xiao Y, Avery P, Chen J, McKenzie C & Osborne L (2012) Ornamental pepper as banker plants for establishment of *Amblyseius swirskii* (Acari: Phytoseiidae) for biological control of multiple pests in greenhouse vegetable production. *Biological Control 63*: 279–286.

Xu X & Enkegaard A (2010) Prey preference of the predatory mite, *Amblyseius swirskii* between first instar western flower thrips *Frankliniella occidentalis* and nymphs of the twospotted spider mite *Tetranychus urticae*. *Journal of Insect Science 10*: 149.

Zavaleta-Mejía E & Gomez RO (1995) Effect of *Tagetes erecta* L. and Tomato (*Lycopersicon esculentum* Mill). Intercropping on some tomato pests. *Fitopatología 30*: 33–45.

How to cite this article: Lopez L & Liburd OE (2022) Can the introduction of companion plants increase biological control services of key pests in organic squash? *Entomologia Experimentalis et Applicata* 170: 402-418. https://doi.org/10.1111/eea.13147