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

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Abstract

Florida 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 (*Bemisia tabaci* Genn., MEAM1; Hemiptera: Aleyrodidae) and the melon aphid (*Aphis gossypii* Glover, Hemiptera: Aphididae) jeopardizes plant development and transmit viruses of economic importance that can cause up to 50% yield loss in ~~organic~~ squash crops. Pesticides are generally used for insect management in ~~conventional~~ squash, but the development of insecticide resistance and their non-target effects are major concerns. ~~Organic growers have limited pest management tactics for insect pest management since pesticides are not allowed.~~ 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, while natural enemies were more abundant when the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) was released alone or together with sweet alyssum. All companion plants used in this study increased natural enemies, but only African marigolds and sweet alyssum ultimately increased biological control activities.

The use of insecticides continues to be a common management tactic used by conventional squash (*Cucurbita pepo* L., Cucurbitaceae) growers against major insect pests such as the melon aphid (*Aphis gossypii* Glover Hemiptera: Aphididae), and the sweetpotato whitefly (*Bemisia tabaci* Genn., MEAM1; Hemiptera: Aleyrodidae), and thrips (Thripidae) (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 tactics does not achieve acceptable levels of pest suppression; therefore, there is a constant need for multi-tactic pest management approaches to successfully suppress key pests in conventional and organic squash production. Although these tactics have been used for decades, they have not been thoroughly investigated.

In Florida, 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 populations changes throughout the year (Mossler & Nesheim, 2011).

Aphids transmit most of the economically important viruses that infect squash, including *Papaya ringspot virus* (*PRSV-W*), *Watermelon mosaic virus* (*WMV*), *Zucchini* *yellow mosaic virus* (*ZYMV*, Potyviridae), and *Cucumber mosaic virus* (*CMV,* Bromoviridae) (Nyoike and 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. ~~In contrast, s~~

Sweetpotato whiteflies transmit the *Cucurbit leaf crumple virus* (*CuLCrV*, Geminiviridae), *Squash vein yellowing virus* (*SqVYV,* Potyviridae), and *Cucurbit yellow stunting disorder virus* (*CYSDV,* Closteroviridae) (McAvoy, 2016; Razze et al., 2016a; Akad et al., 2008). 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, can transmit the virus to healthy plants (Whitfield et al., 2015).

Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae) is an effective predator of the ~~major~~ key pest~~s~~ and disease vector~~s found in Florida squash production, including the~~ sweetpotato whitefly, and ~~various~~ secondary squash pests such as thrips ~~species~~ (Buitenhuis et al., 2015; Kutuk et al., 2016; Xu & Enkegard, 2010). It has been used in several vegetable crops ~~hosts~~ including pepper, cucumber, and eggplant for management of insects and mite pests (Farkas et al., 2016; Stansly & Natwick, 2010; Stansly & Castillo, 2009; Nomikou et al., 2002). 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. ~~Whitefly nymphs were suppressed more than 99 % when *A. swirskii* was present compared with an exponential increase of up to more than 200 nymphs per leaf when *A. swirskii* was absent.~~ The option of targeting two pests with a single natural enemy has positive implications for biocontrol and resembles pest-predator complexes in ~~field~~ natural conditions (Calvo et al., 2015; Messelink et al., 2010). Because *A. swirskii* is a generalist predatory mite species it has the additional capacity to feed on pollen from different plants species (e.g., pepper, cattail pollen, among others), increasing ~~it~~the chance of establishment at early stages of the crop when pests are absent but flowering plants are present (Lopez et al., 2017, Calvo et al., 2015). Despite its wide use on vegetable crops, most studies investigating the performance of *A. swirskii* have been conducted in greenhouse-grown vegetables (Kheirodin et al., 2020; Stansly et al., 2018) and very few have evaluated its performance and establishment in squash crops (Calvo et al., 2015).

~~Nonetheless, there is little information regarding the performance of *A. swirskii* in open-field vegetable crops such as squash (Kheirodin et al., 2020).~~

Companion planting or intercropping involves ~~introducing~~ growing other plants (crop or non-crop plants) within the cropping system together with the cash crop. It has been used as a diversification tactic mostly to increase soil quality, ~~, plant diversity, and~~yield, and ~~enhance~~ promote biological control services by beneficial arthropods (Juventia et al, 2021, Wang, 2012). Insectary plants are used in conservation biological control within vegetable production to provide alternative shelter and food items to beneficial arthropods (Banedes-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 (Jankowska et al., 2012; Wang, 2012; El-Gindi et al., 2005). 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 (Jankowska et al., 2012, Silveria et al., 2009, Gundannavar et al., 2007).

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 (Koptur & Pena, 2018; Wang, 2012, Letourneau, 1990). 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 (Tanga & Niba, 2019; Brennan, 2013; Gontijo et al., 2013; Hogg et al., 2011; Gillespie et al., 2011; Skirvin et al., 2011; Bugg et al., 2008). 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 (Banedes-Perez, 2019).

Squash is considered an excellent candidate for intercropping systems due to its short production cycle (approximately eight weeks) and ease of growing. In addition, ~~B~~biological control agents can be released within squash crops to enhance biological control services. Thus, the goal of this study was to evaluate the potential of three companion plants as a technique for~~of~~ conservation biological control alone and in combination with the release of a generalist predator (*A. swirskii*) as an augmentative biological control technique to determine if there are increases in biological control 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 ~~and~~ to suppress aphids and whiteflies.~~,~~ Finally, we~~and~~ evaluated ~~its~~ the effect of companion plants and *A. swirskii* on viral incidence and yield in organic zucchini squash.

We predict that ~~Non-crop~~companion plants ~~can~~ 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 while 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. ~~Consequently, companion plants within the squash cropping system may have a positive effect on *A. swirskii* populations.~~

~~Thus, the goal of this study was to evaluate the potential of three companion plant species to determine if there are increases in biological control services against key pests, aphids and whiteflies. Furthermore, we determined the role that selected companion plants play in the presence or absence of a generalist predator (*A. swirskii*), for biological control of key insect pest populations, its effect on viral incidence, and yield in organic zucchini squash~~.

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) (29°24'28.0"N 82°08'50.1"W), Citra, Florida). Each year of experiments comprised two squash seasons, fall and spring seasons. 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 (*Cucurbita pepo* L. pepo, Cucurbitaceae), 'Cash Flow' cultivar (Siegers Seed Co., LaBelle, FL), was used as the crop in all experiments. In 2015, African marigold (*Tagetes erecta* L., Asteraceae) cultivar 'Crackerjack' (Stokes Seeds, Buffalo, NY) and cowpea (*Vigna unguiculata* (L.) Walp., Fabaceae) cultivar 'Mississippi Silver' (Urban Farmer, Westfield, IN) were used as companion plants. ~~They were chosen for the experiments due to their effectiveness in repelling some insect species and attracting beneficial arthropods (Hooks et al., 2010). Data collected during the 2015 experiments showed that cowpeas are highly attractive to aphid species and appeared to become a source of aphid infestation in the squash plots. Thus, cowpeas were not used in 2017.~~

In 2017, African marigold was re-used, and cowpea was replaced with sweet alyssum (*Lobularia maritima* (L.) Desv., Brassicaceae) cultivar 'Tall White' (Urban

Farmer, Westfield, IN). ~~Sweet alyssum has been shown to attract predators, parasitoids, and other beneficial arthropods (pollinators) into various cropping systems (Tanga & Niba, 2019; Brennan, 2013; Hogg et al., 2011).~~

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 Inc, Lake City, FL). A rotation of the organic fungicides Regalia® (Marrone Bio Innovations, Davis, CA) and DoubleNickel55® (Certis USA, LLC., Columbia, MD) 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 were used to evaluate the effect of companion plant species or its combination (marigold and cowpeas 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 and 91-cm wide, 1.06-m apart) (Figure 1-A). 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) for insect control; and 5) no companion plant or any type of pest management (control). Spinosad (Entrust®) ~~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), an insecticidal with the active ingredient, potassium salts of fatty acids, was used during the 2017 experiments (Razze et al., 2016b). Additionally, releases of the predatory mite *Amblyseius swirskii* were included in 2017 as a complementary tactic with the use of companion plants. ~~The goal was to investigate if the companion plant species alone or its combination with the inoculated predatory mite *A. swirskii* (marigold or alyssum plus *A. swirskii*) had a significant effect on pest suppression and naturally occurring beneficial arthropods (predators and parasitoids) with the potential to provide additional biological control services in organic squash.~~

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 raise beds (18-cm high and 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 1-B). This type of treatment arrangement helped to determine if companion plants, marigold or sweet alyssum intercropped in organic squash will give additional benefits in terms of pests' suppression compared with augmentative releases of *A. swirskii*. And how would this 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) with vermiculite as bran carrier. Five bran samples (0.5-ml) 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 (~5-m^2) containing squash plants was treated with approximately 20-ml of bran per bed three weeks after planting the squash. Approximately 250 *A. swirskii* motiles were released per square meter. The release rate used was based on the rate recommended for high pest infestations (150–250 mites/m^2, BioBest, 2013, Koppert Biological Systems, 2013).

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 five-week period each season starting three weeks after planting (WAP).~~ the squash in the field for both 2015 and 2017 experiments~~. Viral disease incidence was screened once during the last three weeks of each squash season before ~~crop termination and~~

marketable yield was recorded ~~during the last three weeks of each squash season~~. Beneficial arthropods and pests were also recorded in the companion plants weekly for five weeks following the same sampling protocol used in the squash. ~~viral disease incidence, and marketable yield were monitored in each plot and recorded for the squash and companion plants as described below. A five-week sampling period was conducted every season starting three weeks after planting (WAP) the squash in the field.~~

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

Beneficial arthropods

Beneficial arthropod species including predators and parasitoids ~~Insect and mite predators~~ were recorded weekly using *in situ* counts from six squash and six companion plants (chosen randomly) 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 number of beneficial arthropods observed (Nyoike and Liburd, 2010). In addition, parasitoids and predators were monitored weekly using three 28×23-cm yellow sticky traps (Great Lakes IPM, Vestaburg, MI) per plot in 2015 and two sticky traps per plot in 2017. Sticky traps were left in the field for 48 hours, traps were collected in Ziplock bags and processed in the laboratory. A representative sample from each species was collected and mounted for identification.~~Beneficial arthropod species were also monitored using two clear pan traps (PackerWare®) 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 Co., New York, NY). The detergent solution was refilled, collected, and transported weekly to the laboratory for counting. A representative sample from each species was collected and mounted for identification.

Parasitoids and predators were monitored weekly using three 28×23-cm yellow sticky traps (Great Lakes IPM, Vestaburg, MI) per plot in 2015 and two sticky traps per plot in 2017. Sticky traps were left in the field for 48 hours, traps were collected in Ziplock bags and processed in the laboratory.

In 2017, *A. swirskii* was monitored weekly by collecting three leaves from four squash plants (48 leaves per treatment). One 4-cm diam. leaf discs was taken from each leaf using a cork borer (Cole-Parmer, Vernon Hills, IL) in the laboratory and the number 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 in the pan traps used for beneficial arthropod monitoring. monitored using two clear pan traps (PackerWare®) 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 Co., New York, NY). The detergent solution was refilled, collected, and transported weekly to the laboratory for counting and identification. ~~A representative sample from each species was collected and mounted for 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, ~~I~~immature whiteflies were monitored weekly by collecting three leaves from six squash plants (72 leaves per treatment) ~~in 2015~~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 one 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 Inc. (Elkhart, IN), 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 N was used for sample disruption of ~0.2-g of plant tissue per sample and DNeasy Plant Mini Kits (Qiagen Inc., Germantown, MD) were used for DNA extraction. Apex Hot Start 2X blue master mix (Genesee Scientific Co., San Diego, CA) and *CuLCrV*-specific primers from the DNA-B component (V1324, 5′-TTCTTCTGGTAAAATATGGC-3′ and C2370, 5′-CGACGAGATATGTCAACG3′, Hagen et al., 2008) were obtained from Integrated DNA Technologies Inc. (IDT, Coralville, IA) to direct the amplification of an expected ~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 1-C).

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 PROC 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 PROC 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. *P*-values less than 0.05 were considered significant. All models were fitted using SAS 9.4 (SAS Institute, Cary, NC). Descriptive statistics were used to compare viral incidence among treatments due to the low viral incidence.

Results

A total of 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 recorded included minute pirate bugs (*Orius* sp., Hemiptera: Anthocoridae), big-eyed bugs (*Geocoris* sp., Hemiptera: Geocoridae), lady beetles (Coccinellidae), 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 zucchini squash crops including the melon aphid (*A. gossypii*), sweetpotato whiteflies (*B. tabaci*), thrips (Thysanoptera), melonworms (*Diaphania hyalinata* L., Lepidoptera: Crambidae), and pickleworms (*D. nitidalis* Stoll). 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. since 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< 0.00~~0~~1), with no time or interaction effect. Approximately two times 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 (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). ~~This is equivalent to approximately one-minute pirate bug recorded on every companion leaf sampled from treatments including marigolds alone or mixed with cowpeas.~~ 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 ~~M~~minute pirate bugs ~~collected in yellow sticky traps~~ were ~~lower~~ 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 five to six times 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 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 (spinosad) applications that showed the lowest number of lady beetles. ~~Low numbers of coccinellids were~~

Low numbers of coccinellids were recorded during the fall of 2015 with no significant differences among treatments and no time or interaction effect (Table 1). The numbers of coccinellids collected in the yellow sticky cards in spring 2017 did not differed 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 in soft-bodied arthropods. More long-legged flies were collected in the spring compared with the fall 2015. Significant 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 ($F_{6,18}$= 13.57, P< 0.0001 and $F_{6,18}$= 33.01, P< 0.0001 for spring and fall, respectively), 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 significant differences among treatments for the total number of parasitoids collected ~~using yellow sticky traps~~ ($F_{4,12}$= 51.55, P< 0.0001) in spring 2015. Low parasitoid numbers were found in treatments including Entrust applications and no pest management (~~Figure~~ Table 1~~-A~~). Platygastridae, Encyrtidae, Pteromalidae, Aphelinidae, and Dryinidae were the most abundant parasitoid families collected in spring 2015. Similarly, significant differences among treatments were identified for numbers of parasitoids ~~collected using yellow sticky traps~~ 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 (~~Figure~~ Table 1~~-A~~). Trichogrammatidae, Platygastridae, and Encyrtidae were most abundant than other parasitoid families in fall 2015.

There were significant differences among treatments for parasitoid numbers collected ~~in yellow sticky traps~~ in spring 2017 ($F_{6,18}$= 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 (~~Figure 1-B~~Table 2). Platygastridae and Mymmarydae were the most abundant parasitoid families in spring 2017. The parasitoid numbers ~~collected in yellow sticky traps~~ in fall 2017 did not differed by treatment; nonetheless, more parasitoids were collected in the fall compared to the spring season

(~~Figure 1-B~~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 four WAP (one week after *A. swirskii* release), but predatory mites were recorded five 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 three, six, and seven 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 the melon aphid and the cowpea aphid (*A. gossypii* and *A. craccivora* C.L.Koch). Other species collected included the spirea aphid (*A. spiraecola* Pach), the waterlily aphid (*Rhopalosiphum nymphaeae* L.), the apple-grass aphid (*R. insertum* Walker), the rusty plum aphid (*Hysteroneura setariae* Thomas), the oil palm aphid (*Schizaphis rotundiventris* Signoret), the polygonum aphid (*Capitophorus hippophaes* Walker), and the root aphid (*Tetraneura nigriabdominalis* Sasaki).

Most aphids recorded were winged individuals collected using pan traps. Thus, data from pan traps is 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. There were no differences in winged aphid populations in pan trap catches among the companion plants, however, aAphids were significantly higher on treatments including marigolds alone compared with Entrust (spinosad) and where no pest management was applied (Figure 3-A).

During the same season, the number of winged aphids per squash leaf sampled by *in situ* counts differed among treatments ($F_{4,12}$= 7.36, P= 0.003), with no time or interaction effect. Similarly, the number of wingless aphids sampled by *in situ* counts differed among treatments ($F_{4,12}$= 4.24, P= 0.02), with no time effect, but a significant time-by-treatment interaction was observed ($F_{20,120}$= 3.85, P< 0.0001), such that significant treatment differences were identified four weeks after planting (WAP). High numbers of aphids were found in the organic squash intercropped with cowpeas alone, followed by organic squash intercropped with marigolds mixed with cowpeas. These treatments were significantly higher than marigolds alone, Entrust and where no pest management was applied (Figure 3-B). Significantly higher numbers of aphids were recorded in the leaves of cowpeas planted alone and the leaves of marigolds and cowpeas planted together ($F_{2,6}$= 11.88, P< 0.008), with no time effect, and a significant time-by-treatment interaction ($F_{10,102}$= 7.12, P< 0.0001), such that significant treatment differences were identified four and five WAP. The numbers of aphids inhabiting the marigolds alone remained low during most of the sampling period (Figure 3-CB).

In the fall of 2015, the number of winged aphids sampled using pan traps and *in situ* counts did not differed by treatment, time, and there was no interaction effect

(Figure 3-~~D~~A). ~~The number of wingless aphids in the squash recorded by *in situ* counts differed among treatments ($F_{4,12}$= 9.84, *P*= 0.0009), with no differences over time, but a significant time-by-treatment interaction ($F_{14,60}$= 2.74, *P*= 0.003) was observed, such that significant treatment differences were found four and five WAP. Only the treatment with no pest management showed significantly lower aphid numbers (Figure 3-D).~~ No aphids were recorded by *in sit*u 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 plus the 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 4-A). ~~The number of winged and wingless aphids recorded by *in situ* counts in spring 2017 was not significantly different among treatments, over time, and there was no interaction effect.~~ Hardly any aphids were found inhabiting the companion plants in spring 2017.

In the fall 2017, the number of winged aphids collected by pan traps did not differed by treatment, time, and there was no interaction effect (Figure 4-A). ~~There were significant differences among treatments for the number of winged aphids recorded by *in situ* counts ($F_{6,18}$= 2.78, *P*= 0.004), with no time effect, and a significant time-by-treatment interaction ($F_{30,258}$= 4.71, *P*< 0.0001), such that significant differences were identified at four and eight WAP (Figure 4-B). The squash intercropped with alyssum plus releases of *A. swirskii* and the squash with no pest management showed the~~

~~lowest numbers of winged aphids. There were no significant differences by treatment, and no time or interaction effect for the number of wingless aphids recorded by *in situ* counts in fall 2017 (Figure 4-B).~~

The number of winged and wingless aphids recorded by *in situ* counts in the companion plants differed by treatment ($F_{3,18}$= 346.48, P< 0.0001 and $F_{3,18}$= 6.64, P= 0.001, respectively), with no time or interaction effect. Across treatments, more aphids were found in the marigolds compared with the alyssums (Figure 4-B~~C~~).

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 bioassays 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 since 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 season. Hardly any whitefly adults were collected on yellow sticky traps across treatments (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 5-A). 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 5-A).

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~~1~~±3.44 immatures per disc) and the squash planted next to the cowpeas alone (~~15.51±3.44 and~~ 14.54±3.3~~25~~ immatures per disc~~, respectively~~) (data not shown in figures). Yet, there were no significant differences among treatments, time, or interaction effect for whitefly immatures recorded in ~~leaf discs during~~ 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~~y~~ collected ~~using yellow sticky traps~~ 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 seven WAP. The lowest number of whitefly adults was collected in the treatment where *A. swirskii* was released with no companion plants, while the remaining treatments showed similar whitefly abundances. In fall 2017, there were no significant differences among treatments, and no time or interaction effect for the abundance of whitefly adults (Figure 5-B).

The numbers of whitefly immatures recorded ~~on leaf discs~~ did not differed by treatment or time, but there was a significant time-by-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 three weeks of sampling (0.21±0.14 immatures per disc). Immature numbers increased six and seven 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~~Figure 5-C~~). In fall 2017, slightly more immature whiteflies were recorded in the squash planted next to alyssum alone and alyssum plus *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) (~~Figure 5-C~~data not shown in figures).

Whitefly-transmitted/induced diseases

No significant differences were found among treatments for the averaged SSL index in spring and fall 2015 (Figure 6). Low SSL incidence (1.34±0.08 average index) 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, significant 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 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 2015 (Figure 7-A, B).

There were significant 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 plus the release of *A. swirskii*. The lowest yield was recorded in the control where no pest management was implemented (Figure 7-C, 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 7-C, 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, only a few beetles were collected and 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 different 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 peppers, among other vegetable crops, for management of aphids by the attraction of predatory flies and parasitoid wasps (e.g., braconid wasps) (Brennan, 2016, 2013; Hogg, 2011; Bugg et al., 2008). 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 (Syrphidae) were observed visiting alyssum flowers, but hardly any syrphids were collected by the sampling methods implemented. In contrast, high numbers of long-legged flies (Dolichopopidae) 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 (G. J. Steck, pers. com.) and large populations commonly established throughout the year (Cicero et al., 2017). Generally, flies in the family Dolichopopidae 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 zucchini 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 between 10–35-mm per day) following the release of the predatory could have limited the establishment of *A. swirskii*. Likewise, temperatures below 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 ~~were~~where these trichomes are most abundant. Calvo et al. 2015 and Xiao et al. 2012 ~~it has~~ reported that *A. swirskii* are more likely to establish in crops with glabrous leaves such as peppers or cucumbers~~(Calvo et al., 2015, Xiao et al., 2012)~~. 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 (*A. craccivora*) 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 less 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 the melon aphid. Aphid species like the root aphid, the rusty plum aphid, and the 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 could 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 close proximityproximity.

In 2017, marigolds were observed harboring significantly larger populations of aphids compared to sweet alyssum. Yet, the spill-over effect observed between cowpeas-aphids and squash in 2015 was not observed for marigolds-aphids and 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, repell or intercept insects or the pathogens they vector to reduce theirpest 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., Hemiptera: Aphididae) present in cabbage intercropped with marigolds was 2–7 times 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 could potentially reduce squash viral infection as the aphids' probe and feed on the non-crop plants and deplete their viral inoculum (Mauck et al., 2012; Zavaleta & Gomez, 1995).

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 between primary and secondary veins. 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 ~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 whitflies.

During the squash season in North-Central Florida, it is common to have higher whitefly pressure during the fall compared to the spring season. 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 (~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 cooler temperatures and the presence of *A. swirskii*.~~, and reductions in temperature.~~

In 2017 experiments, the predatory mites appeared to have provided biological control 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, first and second instar nymphs (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. Whiteflies immatures were low the week when the predatory mites were released (four WAP), and low numbers continued to occur for the rest of the experiment, potentially indicating that *A. swirskii* provided some regulation to 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 since 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 high 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 while 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~~-push strategy~~tactic (Juventia et al., 2021, Shrestha et al., 2019). 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 release in the squash as a complementary tactic.

Conclusion

This study aimed to determine, if ~~the~~a combined approach including conservation (companion plants) and augmentative (*A. swirskii* release) biological control techniques ~~introduction of companion plants~~ can increase the biological control 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 ~~in~~ biological control activities. ~~*Amblyseius swirskii* appeared to be successful in suppressing whitefly populations in selected treatments~~

where the predatory mite was deliberately introduced. Nonetheless, it is possible that Tthe combined effects of *A. swirskii* and naturally occurring predators attracted to the companion plants such as *Orius* sp. and long-legged flies, may appeared to have caused the observed 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 zucchini squash cropping system. While marigolds showed potential as a trap crop for aphids, hardly any key pest was recorded in the alyssum, making sweet alyssum an optimal companion plantinsectary plant option 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 U.S. (e.g., Georgia) that are challenged by similar insect pests and disease pressure. Enhancing natural enemies that can suppress insect pests biological control services will reduce the number of insecticide applications, as well as . The production costs can be significantly reduced, and pollinators' and farmers' exposure to toxic chemicals will be minimized. However, there is little research evaluating the performance of generalist predators on squash and open-field production, and further research is required.

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Conflict of Interest

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

References Cited

Akad F Webb S Nyoike TW Liburd OE Turechek W Adkins S & Polston JE (2008) Detection of cucurbit leaf crumple virus in Florida cucurbits. *Plant Disease* 92(4): 648–648.

Banedes-Perez F (2019) Trap crops and insectary plants in the order Brassicales. *Annals of the Entomological Society of America*, 112(4): 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(3): 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(85): 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(4): 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(4): 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(2): 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(3): 307–315.

Farkas P Bagi N Szabó Á Ladányi M Kis K Sojnóczki A Reiter D Pénzes B & Fail J (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(2): 215–220.

Gontijo LM Beers EH & Snyder WE (2013) Flowers promote aphid suppression in apple orchards. *Biological Control* 66(1): 8–15.

Hagen C Rojas MR Sudarshana MR Xoconostle-Cazares B Natwick ET Turini TA & Gilbertson RL (2008) Biology and molecular characterization of cucurbit leaf crumple virus an emergent cucurbit-infecting Begomovirus in the Imperial Valley of California. *Plant Disease* 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.

Hooks CRR Wang K Ploeg A & McSorley R (2010) Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant-parasitic nematodes. *Applied Soil Ecology* 46(3): 307–320.

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(4): 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(1): 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 Roberts PM Chong JH Snyder WE Schmidt & JM (2020) Can generalist predators control *Bemisia tabaci*? *Insects* 11(11): 1–16.

Koptur S Jones IM & Pena JE (2018) The influence of host plant extrafloral nectaries on multitrophic interactions: An experimental investigation. *Plos One* 13(8): e0202836.

Kumar V Xiao Y McKenzie CL & Osborne LS (2015) Early establishment of the phytoseiid *Amblyseius swirskii* (Acari: Phytoseiidae) on pepper seedlings in a predator-in-first approach. *Experimental and Applied Acarology* 65: 465–481.

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(1): 13–17.

Letourneau DK (1990) Mechanisms of predator accumulation in a mixed crop system. *Ecological Entomology* 15(1): 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(1): 1–7.

Martinez L Soti P Kaur J Racelis A & Kariyat RR (2020) Impact of cover crops on insect community dynamics in organic farming. *Agriculture* 10(6): 1–16.

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/ (Accessed: 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: 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(1-2): 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(1): 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(3): 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(8): 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(4): 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): 1–14.

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(6): 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(4): 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(2): 83–111.

Stansly PA & ET Natwick (2010) *Integrated systems for managing Bemisia tabaci in protected and open field agriculture.* In Stansly PA & Naranjo SE (eds.), *Bemisia*: Bionomics and Management of a Global Pest. Springer, New York, pp. 467.

Stansly PA & J Castillo (2009) Control of broad mite, *Polyphagotarsonemus latus* and the whitefly *Bemisia tabaci* in open field pepper and eggplant with predaceous mites. Integrated Control in Protected Crops, Mediterranean Climate. *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(4): 14873–14888.

VSCNews (2020) *Whiteflies impacting Alabama vegetables*. VSCNews, Vegetable and Specialty Crop News. Available at: https://vscnews.com/alabama-whiteflies-vegetables/ (Accessed: 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: 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 & A Enkegaard (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(49): 1–11.

TABLE 1. Mean number (±SE) of predators and parasitoids collected per yellow sticky trap over a five-week period during the 2015 experiments. Back-transformed data are shown ($P \leq 0.05$).

Season	Treatment/Family	Marigold	Cowpea	Marigold +Cowpea	Entrust	No pest management
Spring	Anthochoridae (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 rows followed by the same letters are not significantly different ($P \leq 0.05$).

TABLE 2. Mean number (±SE) of predators and parasitoids collected per yellow sticky trap over a five-week period during the 2017 experiments. Treatments with the same letter are not significantly different (*P*≤ 0.05). 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	Anthochoridae (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 rows followed by the same letters are not significantly different (*P*≤ 0.05).

TABLE 3. Percentage (*n*= number) of samples showing positive results for viral infection during fall 2015.

Treatment	*% PRSV (n[a])*	*% ZYMV (n[a])*	*% WMV (n[a])*	*% CuLCrV (n[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]*n*= 80 (4 per plot) samples used for ELISA assays.
[b]*n*= 40 (2 per plot) samples used for PCR.

TABLE 4. Percentage (*n*= number) of samples showing positive results for viral infection during fall 2017.

Treatment	% Potyvirus group *(n)*	*% CuLCrV (n)*
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)

n= 56 samples (2 per plot) used for ELISA assays and PCR.
Potyvirus group= *PRSV*, *WMV*, *ZYMV*, and *CMV.*

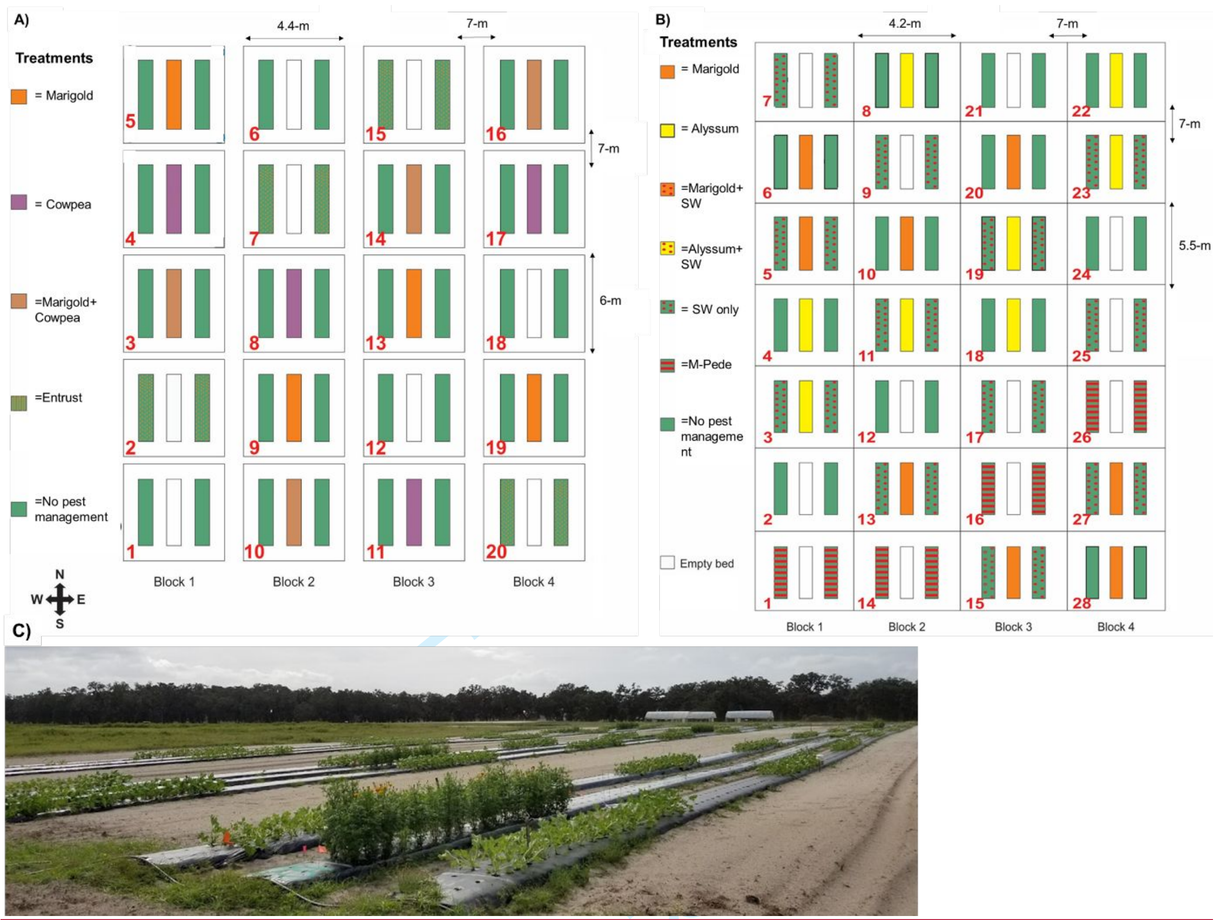

FIGURE 1. Diagram of the experimental design used for the study. A) Experimental layout in 2015 experiments, B) 2017 experiments and C) experimental field in 2015.

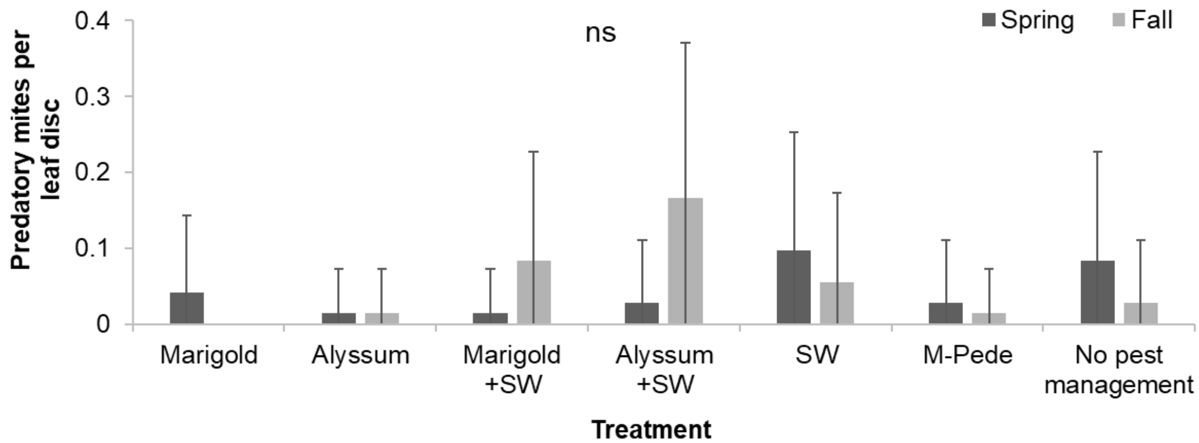

FIGURE 2. Mean (±SE) number of the predatory mites, *Amblyseius swirskii*, recorded per leaf disc (4-cm^2) over a five-week period during spring and fall 2017. Back-transformed data are shown. ns= not significant.

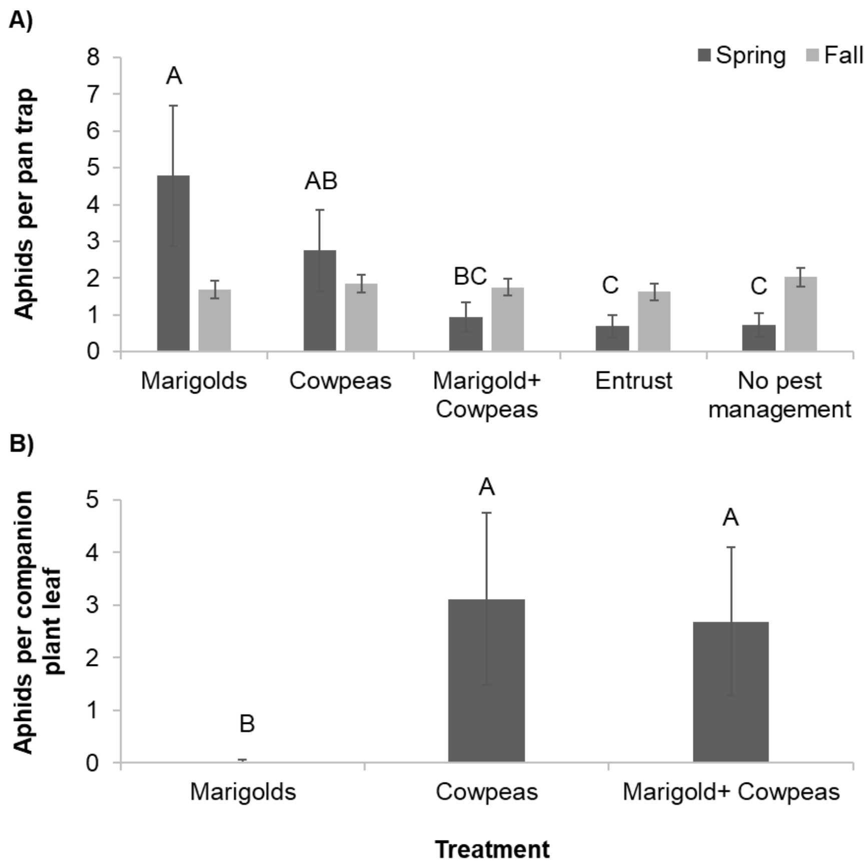

FIGURE 3. Mean (±SE) number of aphids sampled over a five-week period in the 2015 experiments. A) Winged aphids sampled by pan traps in spring and fall~~,~~ and B) ~~winged and wingless aphids sampled by *in situ* counts in the squash in spring, C)~~ winged ~~and wingless~~ aphids sampled by *in situ* counts in the companion plants in spring. ~~, D) winged and wingless aphids sampled by *in situ* counts in the squash in fall.~~ Treatments with the same letter and same capitalization are not significantly different ($P \leq 0.05$).

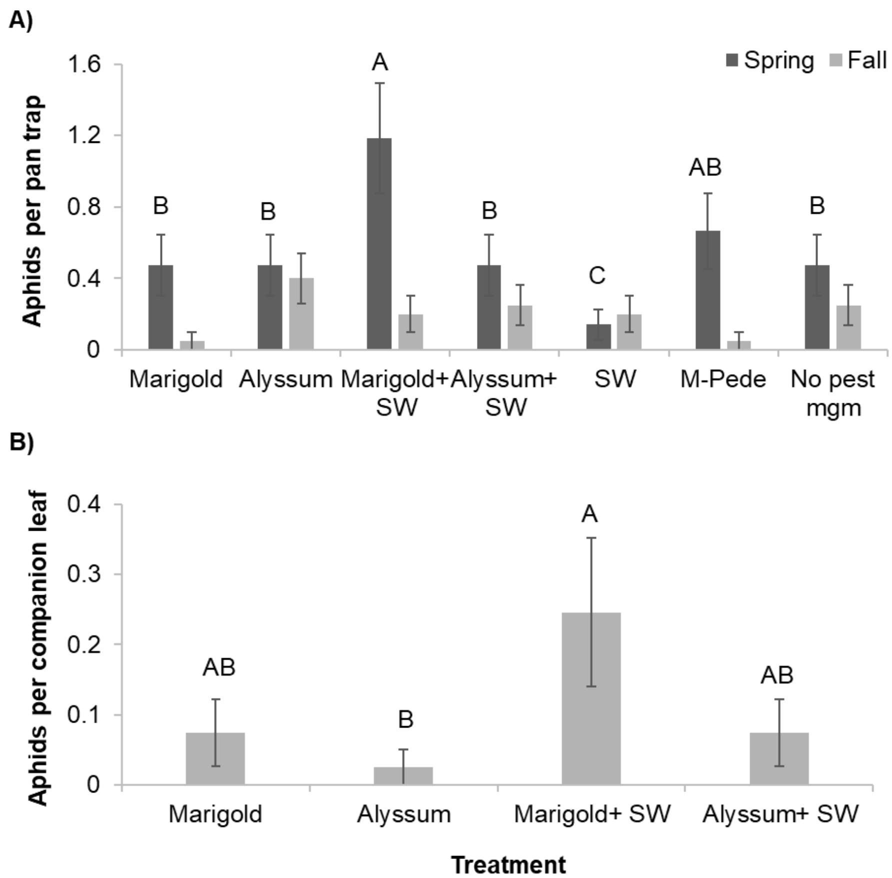

FIGURE 4. Mean (±SE) number of aphids sampled over a five-week period in the 2017 experiments. A) Winged aphids sampled by pan traps in spring and fall and~~,~~ B) ~~winged and wingless aphids sampled by *in situ* counts in the squash in fall, C)~~ winged ~~and wingless~~ aphids sampled by *in situ* counts in the companion plants in fall. Treatments with the same letter and same capitalization are not significantly different ($P \leq 0.05$).

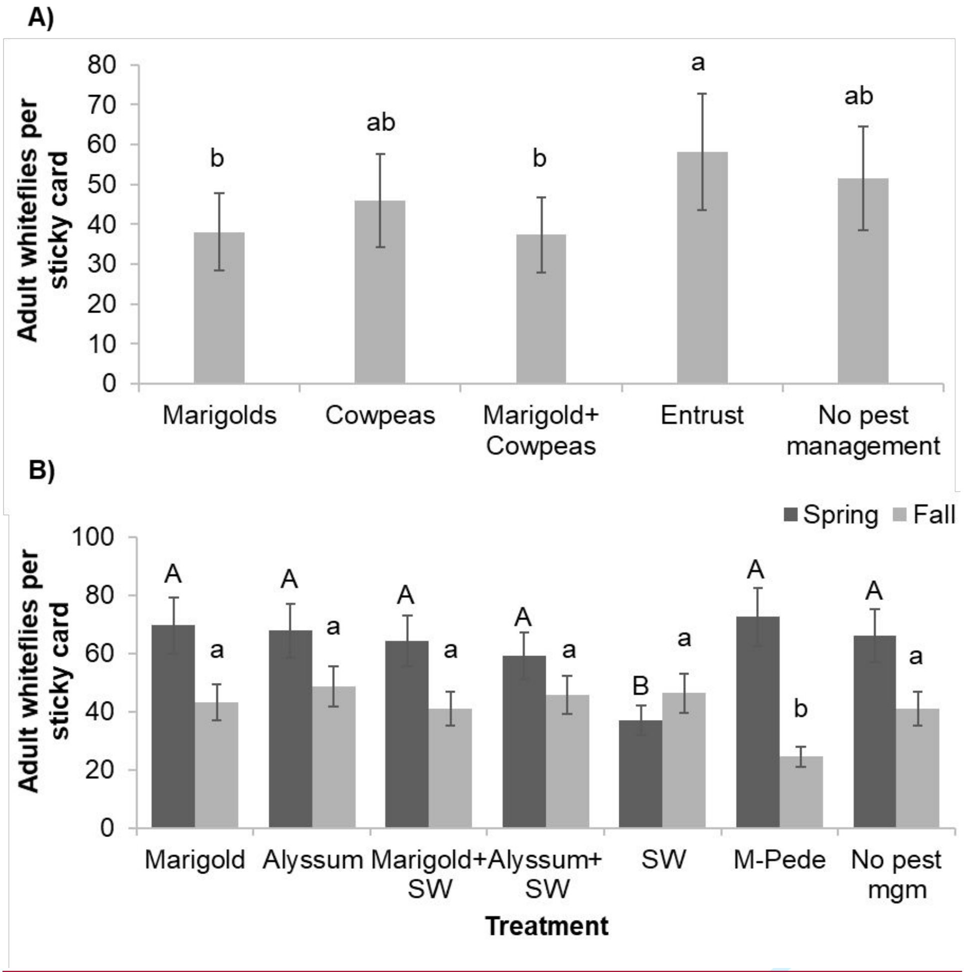

FIGURE 5. Mean (±SE) number of whiteflies sampled over a five-week period in the 2015 and 2017 experiments. A) Adult whiteflies sampled by yellow sticky traps in fall 2015 and B) adult whiteflies sampled by yellow sticky traps in spring and fall 2017. Treatments with the same letter and same capitalization are not significantly different (*P*≤ 0.05).

Mean (±SE) number of whiteflies sampled over a five-week period in the 2015 and 2017 experiments. A) Adult whiteflies sampled by yellow sticky traps in fall 2015, B) adult whiteflies sampled by yellow sticky traps in spring and fall 2017, C) immature whiteflies recorded in squash leaf discs in spring and fall 2017. Treatments with the same letter and same capitalization are not significantly different (*P*≤ 0.05). ns= not significant.

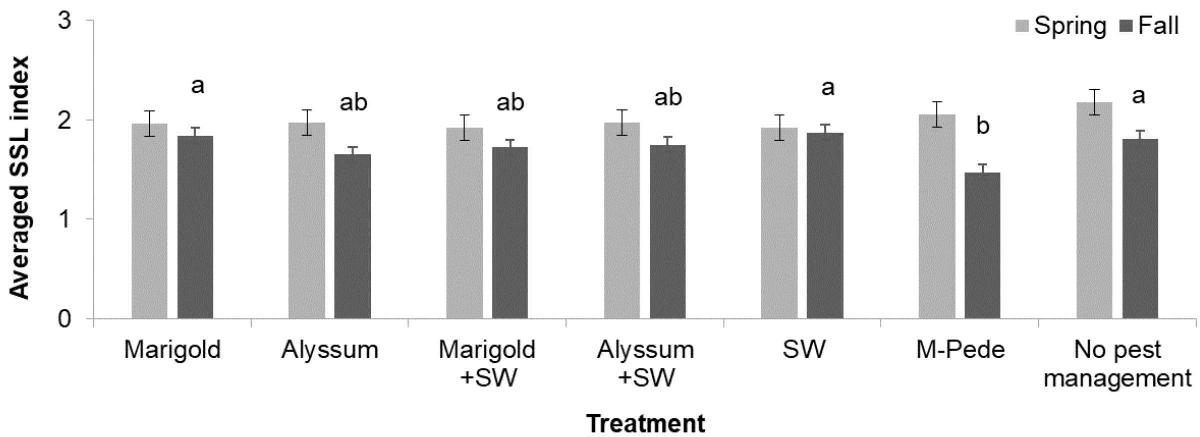

FIGURE 6. Averaged Squash silverleaf (SSL) disorder index (±SE) rated in spring and fall 2017. Treatments with the same letter are not significantly different ($P \leq 0.05$). Back-transformed data are shown.

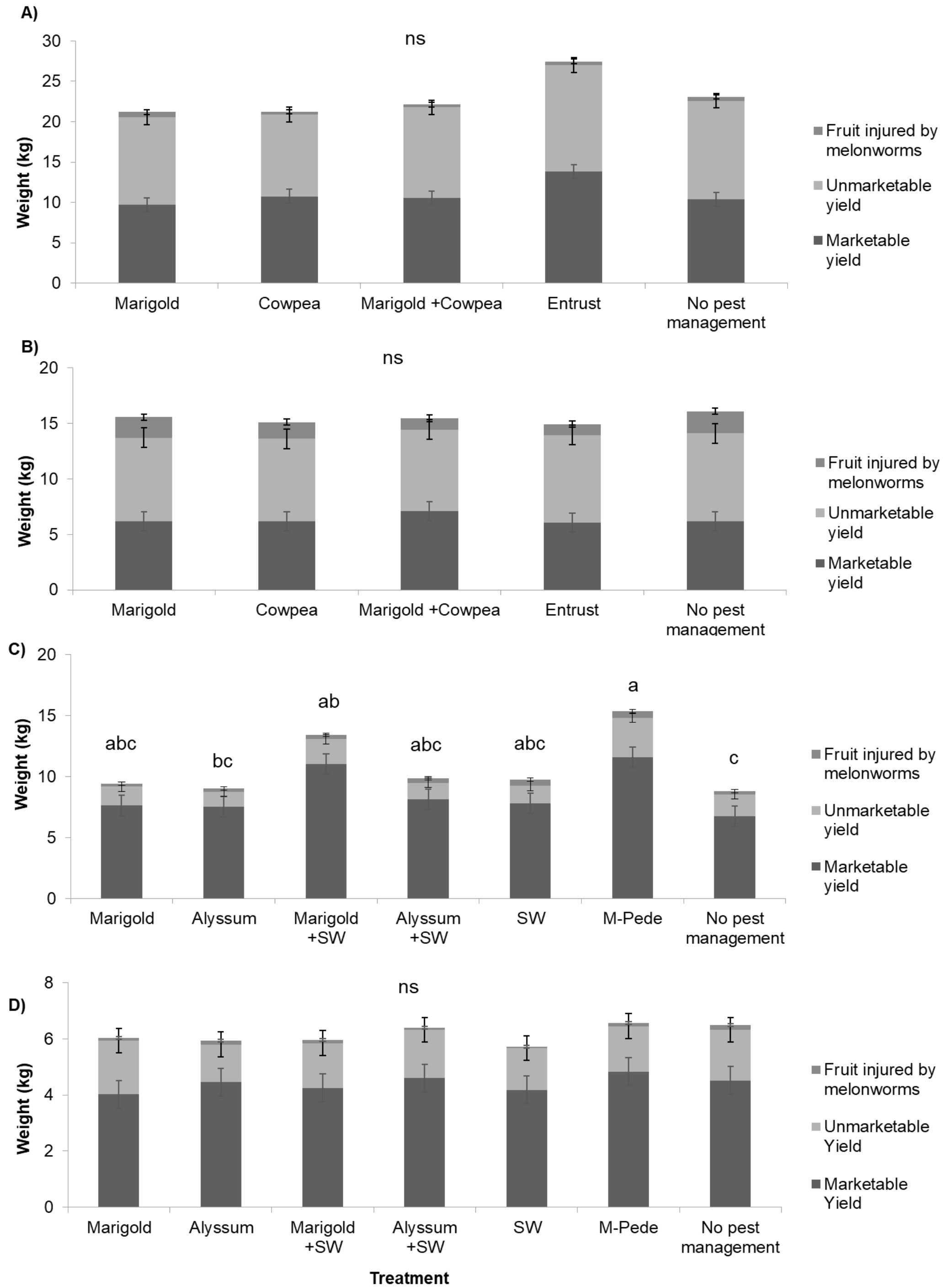

FIGURE 7. Total marketable yield, total unmarketable yield, and total fruit injured by pickleworms (±SE) harvested per treatment in A) spring 2015, B) fall 2015, C) spring 2017, and D) fall 2017. Treatments with the same letter are not significantly different ($P \leq 0.05$). Back-transformed data are shown. ns= not significant.

Letter to Reviewers

Original Article, Article type: Regular paper

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

Lorena Lopez, Oscar E. Liburd

We thank the reviewers for their constructive critique since their comments improved the quality of our manuscript. Here we present our responses to each point.

Reviewer: 1

Line 47: "and thrips..." the name of the species if possible.

ANSWER: The thrips group was deleted from the paragraph since it is not within the scope of the study nor a major pest issue in cucurbit in Florida (Ln 51).

Line 48-50: "However... pest resistance... organism" references needed here to support this statement.

ANSWER: Included as suggested (Ln 53).

Line 52 "... used for decades" since when, and where and how? Please give the year in general, and some examples if possible.

ANSWER: The phrase was modified for clarification (Ln 56-59). Examples of the use of cultural management techniques are included further in the introduction section.

Line 84-93: "Companion planting...insectary plants...natural enemies..." more recent references (2013-2021) on this issue and related fields such as banker plant system should be integrated briefly in this paragraph (if possible) for a better understanding the current condition of this biocontrol tactics, to ensure if the method discussed in this paper is a promising one in the future.

ANSWER: More recent references were included as suggested (Ln 105-111). Additionally, the paragraph was modified to better explain the role of companion planting and insectary plants in the study. Banker plant systems were not mentioned in the introduction section since this system works very differently from the methods used in our study. Banker plants systems imply rearing and establishment of natural enemies on insectary plants before their release into the crop. This can be used as a method to augment or inoculate natural enemies; however, this was not the method implemented during the experiments.

Line 114-120: "Two year-round experiments...the spring season... experimental area..." The GPS coordinates for the experimental areas should be provided.

ANSWER: The experiments were conducted in the same area for both years and the coordinates were included as requested (Ln 162-163).

Line 154: "Entrust was applied twice..." better change to "Spinosad (Entrust (R) was appled twice..." to avoid confusion.

ANSWER: The phrase was changed as suggested (Ln 204).

Line 266: The first appearance of "pickleworms" should be followed by "Diaphania nitidalis Stoll (Lepidoptera: Crambidae)" for better understanding.

ANSWER: The scientific name and family was included as requested (Ln 346).

Reviewer 2

General comments

ANSWER: The conservation (companion planting) and augmentative (release of predatory mites) biological control techniques used during the study aimed to increase the suppression of key pests in squash and important viral diseases. Our decision to show the findings regarding pests, natural enemies, and viral incidence together is grounded on the evaluation of the effects of these techniques over disease incidence as one of the objectives of the study. Dividing the manuscript into two documents would limit the interpretation and understanding of our findings, as well as the potential application of these pest management techniques. Therefore, we kept the results of viral incidence in the present manuscript.

Information about the performance of the predatory mites in open-field cropping systems is very limited. There are only a few studies evaluating their establishment and suppressive effect in open field-grown crops and the scientific community as well as the growers are very interested to find out more information about the performance of these predatory mites under these conditions. Growers in Florida expressed their interest in learning more about the effectiveness of predatory mites to suppress key pests, and how to release them in cucurbit crops including squash. Therefore, although the results about *Amblyseius swirskii* are not striking, we believe that our results regarding this predatory mite should be included in the manuscript since they represent a starting point for future studies by demonstrating the challenges of establishing biological control agents (predatory mites) in open squash fields.

Regarding the objectives in the introduction part, the last paragraph on this section was modified to clearly state the types of pest management that were evaluated, as suggested by the reviewer (Ln 131-139).

Initially, when the statistical analyses were conducted, year and season were included as a fixed effects to evaluate their effect on the data. Significant differences between years and among season were identified, and that is the reason why the analyses shown in the present manuscript are shown by year and season. These differences may be explained not only by the different treatments evaluated between years but also by changes in the population dynamics of the pests and natural enemies across seasons. Therefore, the combination of treatments suggested is not feasible due to statistical differences across trials.

There seems to be a widespread misconception in Florida about the use of Entrust (spinosad) as the default option to control almost any soft-bodied pest in vegetable crops. This has led to many growers using Entrust to control whiteflies and many agents recommending it for the same use. However, this product is not labeled nor is effective against this pest. The reason why, we believe is important to make clear that Entrust is not an appropriate tool against whiteflies and compare it with the use of M-Pede®, which can successfully suppress them.

Aphid data was reduced substantially, which was the longest subsection in the results. We focused on winged aphids for most of the aphid data since they were the most abundant throughout the experiments. Monitoring winged aphids are of great significance in Florida where winged aphids are produced all year long due to the warm climate. They represent the colonizing stage of the species and can show accurate data about colonizing (species that use squash as host) and non-colonizing aphid species (aphids that do not use squash as host but may feed on them). By reducing the data shown about aphids we were able to reduce the number of graphs included in the manuscript as recommended. Multiple graphs were removed from the figures, including all the ones showing wingless aphid data. In addition, Figure 1 showing parasitoid numbers was deleted and the parasitoid data was included in Tables 1 and 2 together with the predator's data. Most graphs showing not significant data were removed.

Despite recording and identifying natural enemy species in pan traps, yellow sticky traps, and *in-situ* counts, only the natural enemy data from yellow sticky traps are shown in the manuscript. Most natural enemies were collected using yellow sticky traps except for *Orius* spp., reason why only *Orius* abundance was included in the results based on *in-situ* counts. It was never an objective of the study to evaluate nor compare trapping methods; nonetheless, we used multiple methods to cover the diversity of arthropods that we expected to find in the squash cropping system.

The missing references in the introduction section were added as suggested.

The methodology was modified to clearly state that other natural enemies besides predatory mites were sampled, as suggested (Ln 388-389). The number of natural species as well as the proportions based on their guild (predator, parasitoid, plant feeders) were already included in the results section.

We mentioned in the results section the number of arthropod species we collected overall the study; however, we consider that calculating richness and evenness biodiversity indexes are not an ideal method to interpret our findings. This is because one of the main goals of the study was to evaluate if the pest management techniques utilized could increase natural enemy populations that subsequently provide biological control services in the squash. Thus, we focused on the pests and natural enemies that played active roles in the squash cropping system. This included key pests of the squash and predators and parasitoids that could suppress those pest species. Using biodiversity indexes including all arthropod species collected (regardless of their abundance or role) could lead to overestimating the effect of the companion plants in the cropping system.

Specific comments

L30: it would be interesting to have just a value in USDA of the impact of those pests on the production.

ANSWER: Unfortunately, we haven’t been able to find estimates about the effect of viral diseases in the squash from the USDA but an estimate calculated at the University of Georgia was included in the abstract (Ln 30) and introduction (Ln 63). The reference was included in the reference list as well.

L32 – 33: be careful, I disagree, many solutions are existing today to reduce pest pressure in organic but they are more complicated to implement than pesticides. I would take that sentence out.

ANSWER: Phrase deleted as suggested (Ln 33-34).

L40: it would be good to make a concluding Remarque at the end of the abstract. Finishing with only the results is poor.

ANSWER: Concluding phrase included as recommended (Ln 42-44).

L50: same comment as L32 – 33.

ANSWER: The phrase was modified for clarification (Ln 54).

L50 – 52: refs

ANSWER: Reference included as required (Ln 53).

L55 – 56: ref

ANSWER: The reference does not apply to this sentence since it is stating the need of the field (Ln 56-59).

L64: ref

ANSWER: Reference included as required (Ln 64-65).

L77 – 79: no need to detail the results of the other study. Saying that this natural enemy is efficient is enough.

ANSWER: The detailed phrase: "Whitefly nymphs were suppressed more than 99% when *A. swirskii* was present compared with an exponential increase of up to more than 200 nymphs per leaf when *A. swirskii* was absent" was deleted and the previous phrase was modified (Ln 90-92).

L80: ok, but you are studying aphids and not thrips. Does this predator has been proved to have any effect on aphids?

ANSWER: Not, really. *Amblyseius swirskii* may probably eat 1st instar aphids but aphids are not the usual prey for this mite. However, thrips are an important prey for this predatory mite and even though thrips are not a major pest in squash, they are usually present in the leaves of the squash (as non-colonizing insects) and they were present in the companion plants. Their presence may play an important role to maintain this predatory mite and predatory insects (e.g., minute pirate bugs) around the squash, reason why we mentioned the importance of pest-predator complexes (Ln 92).

L102: ref

ANSWER: No reference was included since this paragraph refers to the stated hypothesis of the study (Ln 144-150).

L106: you don't make any assumption on why companion crops can have an effect on the presence of the predatory mite. It is missing. You are detailing the effect on other types of natural enemies but these natural enemies need pollen and nectar to develop, and that's the reason why they develop well when there are companion crops. Here, with mites, I am not sure that the argument is valuable.

You need to detail why you make the hypothesis that companion crops could increase the presence of predatory mites.

ANSWER: This is a great point. *Amblyseius swirskii* can feed on pollen from different crop and non-crop plants (e.g., ornamental peppers, sweet alyssum); reason why we believed companion plants could promote their establishment. We have clarified the point addressed by the reviewer and the introduction section was modified in Ln 95-102.

L26 – 128: this is introduction part.

ANSWER: This phrase was deleted from the materials and section as suggested since it is already mentioned in the introduction section (Ln 174-176).

L128 – 130: this is result part.

ANSWER: The phrase was deleted as suggested since it is already mentioned in the results section (Ln 176-179).

L133 – 135: this is introduction part.

ANSWER: The phrase was added to the introduction section and deleted from the methods section as suggested (Ln 182-184).

L154: don't forget to add an ® to the name of the product Entrust ®. Same L156.

ANSWER: Most scientific journals only require including the ® for the first mention of the pesticide brand name, reason why I only included in the materials and methods section.

L154: it would have been interesting to have a figure with the study design + some pictures. Sometimes a picture is worth 1000 words!

ANSWER: A diagrammatic figure of the experimental layout and pictures of the 2015 and 2017 experiments were included as suggested.

L161 – 164: this is introduction part.

ANSWER: The paragraph was deleted since the goal of the study was already included in the introduction section (Ln 212-216).

L178: same as for 2015 design, it would be nice to have a figure with the study design + some pictures.

ANSWER: A diagrammatic figure of the experimental layout and pictures of the 2015 and 2017 experiments were included as suggested.

L193 – 194: I am not sure to understand how many samplings in total were done on both sampling years. Could you be more precise on that specific point?

ANSWER: Details about the number of sampling events were included as suggested (Ln 244-252)

L199: why did you change the method between 2015 and 2017?

ANSWER: Three reasons for that. First, we wanted to suppress aphids and whiteflies using sustainable practices, including conservation biocontrol, but these pests are suppressed by different complexes of natural enemies in FL. The predators of aphids are not usually the same that feed on whiteflies and vice versa and based on the season (spring or fall) both natural enemy complex and the pest pressure varies. The sole use of companion plants did not seem to be enough to suppress both pests in the squash. Thus, we wanted to complement the use of companion plants that promotes conservation biocontrol with another pest management technique (augmentative biocontrol) to increase the chances of pest suppression. Second, growers expressed interest in using biocontrol agents in their squash but had no clear guidelines on how to do so. The release of lady beetles and minute pirate bugs can be very expensive, and research has shown unclear results on their suppressive on whiteflies. Third, we decided to use the predatory mite *Amblyseius swirskii* because it is a predatory mite that can establish early in the presence of flowering plants because it feeds on pollen and it can feed on other prey such as thrips, which are always around the squash despite not being considered a major pest in this crop most of the time.

L310: is it not $P<0.001$ instead of 0.0001?

ANSWER: The value was modified as recommended (Ln 397).

L317: can you support what you say with statistical results? Every time you say that there is a significant difference, then you need to specify the statistical result as you did L314. This needs to be applied in the entire result part.

ANSWER: The phrase was deleted to avoid misinterpretation (Ln 405-407).

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