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## ORIGINAL ARTICLE

# Effects of intercropping marigold, cowpea and an insecticidal soap on whiteflies and aphids in organic squash

# Marice Lopez 💿 🕴 Oscar E. Liburd 💿

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

#### Correspondence

Oscar E. Liburd, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA. Email: oeliburd@ufl.edu

#### Abstract

The silverleaf whitefly MEAM1 (Bemisia tabaci) Gennadius (Hemiptera: Alevrodidae). the cowpea aphid (Aphis craccivora Koch), the green peach aphid (Myzus persicae Sulzer) and the melon aphid (Aphis gossypii Glover) Hemiptera: Aphididae are major insect pests of squash (Cucurbita pepo L.), causing significant yield losses of up to 80% in Florida. We hypothesized that intercropping African marigold (Tagetes erecta L.) and cowpea (Vigna unguiculata L. Walp.) with zucchini squash will encourage an abundance of beneficial arthropods and increase cultural and biological control against these key insect pests by significantly reducing their populations and consequently improve yields. In a 2-year field experiment, five treatments were evaluated in a randomized complete block design consisting of four replicates. Three diversified cropping treatments were implemented: (1) intercropping squash with marigold, (2) intercropping squash with cowpea, (3) intercropping squash with marigold and cowpea (mixed). These treatments were compared with an organic grower standard insecticide, (4) M-Pede (potassium salts of naturally derived fatty acids) sprayed on monocropped squash and (5) monocropped squash with no pest management (control). Results indicated that squash intercropped with marigolds accounted for the highest marketable yields. Squash intercropped with cowpea had marginally high yields but attracted the highest densities of aphids. M-Pede did not increase squash yields, but reduced aphids, whiteflies and associated squash silverleaf (SSL) disorder ratings and showed no deleterious effect on populations of natural enemies. We conclude that a mixture of marigold and cowpea could be used to suppress pests by increasing beneficial arthropod diversity while enhancing marketable yields of organic squash. M-Pede could be used as a last resort when experiencing high hemipteran pressure.

#### KEYWORDS

aphids, Bemisia tabaci, Cucurbita pepo, organic agriculture, Tagetes erecta, Vigna unguiculata

## 1 | INTRODUCTION

In the United States, approximately 15,000 ha of squash (*Cucurbita pepo* L., Cucurbitaceae) are harvested for the fresh market with a value of \$207.6 million USD (Vegetables, 2023 Summary). Florida

comes second to California in the production of squash with an annual value of \$45.5 million USD, 770,000 CWT, and 2800 harvested hectares in 2022 (Vegetables, 2023 Summary). The traditional system of growing squash commercially using frequent applications of conventional pesticides is unsustainable and contributes to a reduction

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in arthropod diversity (Altieri et al., 2009; Welch & Harwood, 2014), whereas crop diversification provides essential refugia for pollinator species and natural enemies (Guzman et al., 2019). A more sustainable and ecologically friendly system of growing squash is needed to reduce growers' reliance on conventional pesticides.

The silverleaf whitefly (*Bemisia tabaci* MEAM1; Hemiptera: Aleyrodidae) is the key insect pest of squash in Florida. Silverleaf whiteflies induce direct and indirect injury, which account for as much as 35% of losses in squash, annually (Little et al., 2017). The feeding of nymphal whiteflies induces the squash silverleaf (SSL) disorder, an economically important physiological disorder that affects summer squash in Florida and other semi-tropical areas in the world (Yokomi et al., 1990). Nymphs feed on the phloem sap with their needle-like mouthparts and leave openings where air flows into the layers of the leaves, causing the silvered appearance (Costa et al., 1993; Jimenez et al., 1994). These symptoms first appear on the primary veins nearest to the petiole. They gradually spread to secondary veins and eventually cover the entire upper leaf surface.

Several aphid species, including the melon aphid (*Aphis gossypii* Glover), the cowpea aphid (*Aphis craccivora* Koch), and the green peach aphid (*Myzus persicae* Glover; Hemiptera: Aphididae) lower the plant's vitality by feeding on the phloem and removing photosynthates (Rathore & Tiwari, 2014). In response, squash release plant defensive chemicals that can inhibit growth and fruit production, affecting economic yields (Kappers et al., 2011). Aphids and whiteflies also transmit several viral diseases in squash and other cucurbits in Florida (Little et al., 2017). Common symptoms of viral diseases in squash include crumpled leaves, malformed fruit, and stunted plants (Blua & Perring, 1989; Martini et al., 2011; Nyoike et al., 2008).

Diversified cropping is practiced worldwide for a plethora of benefits including maximizing productivity, attracting pollinators and decreasing pest incidence (Huss et al., 2022; Rosa-Schleich et al., 2019). Intercropping, in particular, has consistently proven to be less favourable to pest invasions by disorienting pests from host plants (Altieri & Letourneau, 1982; Chand & Sharma, 1977; Luik et al., 2000; Thomine et al., 2020). Intercropping interrupts the visual orientation of insects from the host plant and can introduce plant chemical volatiles that interfere with olfactory-driven host-finding mechanisms (Andow, 1991; Smith & McSorley, 2000). Although intercropping is widely adopted in organic vegetable production systems, very few studies have documented the benefits of managing pests of squash (Kopittke et al., 2012; Lopez & Liburd, 2022).

African marigolds (*Tagetes erecta* L., Asteraceae) have been used as companion plants and cover crops to increase soil quality and enhance beneficial arthropod communities in diversified cropping systems (Bakshi & Ghosh, 2022; Hooks et al., 2010; Zavaleta-Mejia & Gomez, 1995). Intercropping marigold has been shown to suppress pests on the cash crop by increasing biological control activities (lamba & Teksep, 2021; Lopez & Liburd, 2022; Silveira et al., 2009; Souza et al., 2019). Cowpea (*Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae)) are used worldwide for their nitrogen-fixing properties; however, the extrafloral nectaries (EFN) in cowpea have been shown to enhance the control of aphids and whiteflies by attracting natural enemies such as *Orius tristicolor* White (Hemiptera: Anthocoridae), *Trichogramma* (Hymenoptera: Trichogrammatidae) wasps, lady beetles and spiders (Borkakati et al., 2019; Kopittke et al., 2012; Letourneau, 1990; Wu et al., 2011). Extrafloral nectaries offer up to 70% more sugar than other floral nectars found in most flowers (Kuo & Pate., 1985).

For decades, farmers habitualized the use of broad-spectrum conventional pesticides as the primary means of managing softbodied insects in cucurbits. However, the continuous application of these agrochemicals is responsible for creating imbalances in the beneficial arthropod community, inducing pest resistance, contamination of water systems and changing the biogeochemical nature of the soil (Meena et al., 2020; Sanchez-Bayo, 2021; Tudi et al., 2021). Insecticidal soaps provide control against soft-bodied insects while having rapid degradation and low mammalian toxicity (Henn & Weinzierl, 1989). M-Pede ([Potassium salts of naturally derived fatty acids] Gowan Company) is labelled for organic use and is a commercially available insecticide, miticide and fungicide. It works through contact by desiccating the insect cuticle (Henn & Weinzierl, 1989). M-Pede is used as a last resort when other cultural and biological tactics have proven to be insufficient in organic systems. While the efficacy of M-Pede has been demonstrated under greenhouse conditions, few studies investigated its effects in an organic field setting.

This research evaluates complementary integrated pest management (IPM) strategies to study the direct effects of marigold and cowpea as intercrops, and M-Pede, a pesticide labelled for organic use, on squash marketable yield and on the population dynamics of insect pests and beneficial arthropods. The use of these cultural and chemical control techniques presents a promising alternative to conventional farming practices, given their holistic effects on the beneficial arthropod community and the environment.

#### 2 | MATERIALS AND METHODS

#### 2.1 | Site

The experiment was conducted in an organic plot at the University of Florida's Plant Science Research and Education Center (PSREU), in Citra, FL (29°24′26.45″, 82°9′48.80″) in the fall of 2016 and 2017. In 2016, the experiment began in late October. In 2017, the experiment was delayed until early November due to Hurricane Irma.

#### 2.2 | Field setup and experimental design

The field where the experiment was conducted was certified as USDA Organic in compliance with the National Organic Program. The field was divided into 20 plots, each measuring 7 m wide by 6.7 m long. Each of the plots had two black polyethylene film mulch beds, each bed was 1 m wide, and raised 30 cm from the ground. There were buffer zones 4.57 m from N-S and 4.6 m from E-W between each plot. Each bed received two drip irrigation lines. The irrigation was JOURNAL OF APPLIED ENTOMOLOGY

supplied at 0.89L/min/30m. For every 30min, 1212L of water/ha were supplied. Three to four 30 min application cycles were irrigated depending on the moisture content in the soil and the plants' stress.

The field was organized into five treatments and replicated four times in a completely randomized block design (Figure 1). The treatments consisted of (1) zucchini squash (Cucurbita pepo L., Cucurbitaceae) cultivar 'Cashflow' (Syngenta) intercropped with African marigolds, T.erecta L. (Asterales: Asteraceae) variety 'Crackerjack' (Stokes Seeds); (2) zucchini squash intercropped with cowpea, V. unguiculata (L.) Walp. (Fabales: Fabaceae) cultivar 'Mississippi Silver' (Urban Farmer); (3) zucchini squash intercropped with African marigolds and cowpea (denoted as 'mixed' treatment); (4) monocropped zucchini squash planted on each side of both beds treated with an application of 1.5% solution M-Pede (potassium salts of naturally derived fatty acids, 59 mL/ha) and (5) untreated zucchini squash planted on each side of both beds (designated as control receiving no pest management). African marigolds and cowpea have longer developmental periods than squash; therefore, companion plants were planted in early September 2016 and in mid-September 2017, approximately 3 weeks before planting the squash to synchronize flowering times with the crop. Each bed consisted of two rows made up of 20 holes/row. Seed bunches of either marigold (30–50 seeds) or cowpea (5–10 seeds) were planted on the outer rows of each bed. Twenty individual squash plants were planted on the inner rows of each bed. Companion plants and squash plants were spaced 61cm apart in a staggered pattern to reduce overcrowding.

# 2.3 | Sampling and monitoring for diseases and disorders

For both years, insect sampling and monitoring for diseases and disorders began 3 weeks after the squash was planted and continued weekly for 5 weeks in 2016 and 6 weeks in 2017.

#### 2.3.1 | In situ counts

In situ counts of adult whiteflies, aphids and natural enemies were taken using the leaf-turn method (Nyoike & Liburd, 2010). Three of the middle-outer leaves of six squash plants per plot were gently turned and counts were made for the number of adult whiteflies, alate aphids, predators and parasitoids per bed.

#### 2.3.2 | Evaluation of immature whiteflies

Immature whiteflies were monitored through destructive sampling by randomly collecting four squash leaves, from the middle stratum of the plants, per plot. Leaves were placed into 1-quart Hefty® Storage plastic bags, stored in a cooler and brought to the Small Fruit and Vegetable IPM Laboratory in Gainesville, FL for processing. Four leaf discs were taken from each leaf using a cork borer measuring 3.14 cm<sup>2</sup> in diameter, where discs were punched halfway between the mid-vein and leaf edge, and halfway between the petiole and leaf tip (Gould & Naranjo, 1999). The number of immature whiteflies per leaf was recorded under a Leica MDG41 stereomicroscope (Leica Microsystems).

#### 2.3.3 | Diseases and disorders

Squash silverleaf (SSL) disorder was monitored by randomly selecting six plants from the inner row of each bed and scoring them with an arbitrary scale adapted from Yokomi et al. (1990). Plants were scored from 0 to 5, where 0 was a healthy plant displaying no silvering, and 5 was a completely silvered plant. Disease incidence was monitored by recording the number of squash plants per plot showing viral symptoms.





Trapping

2.3.4

2.4

## 455

## Unbaited Pherocon AM yellow sticky traps (YST; Great Lakes IPM) were used to monitor for whiteflies and parasitoids. Two sticky traps back-transformed data. were deployed per plot (Figure 1) and left in the field for 1 week during the squash growing season. The traps were placed diagonally RESULTS across from each other, inside the rows and were adjusted weekly to 3 match the growth of the plants. To preserve the insects, traps were Whiteflies covered with Glad® ClingWrap (Glad Products Company) plastic 3.1 wrap and brought to the laboratory to be processed under a stereomicroscope. Simultaneously, aphids and predators were monitored 3.1.1 using clear plastic pan traps (PackerWare®) hung on the middle to lower level of tomato cages. The placements and orientation were similar to the YST, in alternating locations within the plots. Two pan traps were deployed for every replicated treatment every week and collected after 48h. Aphids and predators were preserved in 70% ethyl alcohol and were processed under a stereomicroscope. **M-Pede** application

Two applications of M-Pede (Gowan® Company) insecticidal soap were applied to the treatment at 3 WAP (weeks after planting) and at 5 WAP. Applications were made on the day of trap deployment (2 days before monitoring for insects and observing for diseases and disorders) at a rate of 59 mL/ha (1.5% v/v solution) using a 15-L (4gal) calibrated backpack sprayer (model 10207, SOLO® 425) with a hollow cone nozzle at 60-PSI. Spraying was conducted during the morning hours when temperatures were below 29.4°C (85°F) and when pollinators were less active.

#### 2.5 Marketability

Zucchini squash was harvested three times per week and separated and weighted based on its marketability. Marketable squash fruit was between 10 and 20 cm in length and without any discernible injury. Fruit that was deformed, larger than 20 cm, rotted and/or with melon worm damage was noted as unmarketable.

#### 2.6 **Statistical analysis**

Data for whiteflies, aphids, natural enemies, SSL disorder and marketable yield were analysed. Whitefly, aphid and natural enemy counts were square-root transformed to satisfy model assumptions. Data were separated by year and analysed by repeated measures analysis of variance with generalized linear mixed models (ANOVA; PROC GLIMMIX) using either a Normal distribution or Poisson distribution with a log link function (SAS Institute Inc.) and Kenward-Rogers correction for degrees of freedom adjustment. The models examined treatment, time and the interaction between treatment as fixed effects and blocking as random effects. All data were

considered significant when  $p \le 0.05$ . Significant differences between treatment means were analysed using Tukey's multiple comparison tests. Reported means and standard errors (SE) are from

#### In situ counts

In 2016, there were 51%, 62% and 70% fewer adult whiteflies observed on zucchini in the cowpea, mixed and M-Pede-treated plots compared with the marigold intercropped plots, respectively (F=7.19; df=4, 200; p < 0.0001), but adult whitefly counts on zucchini in these plots were not significantly different to the control plots (Figure 2a). Whitefly numbers were highest 3 WAP and decreased significantly in the following weeks (F = 72.06; df = 4, 200; p < 0.0001). Densities also differed between treatments and time (F=4.60; df=4, 200; p<0.0001). In 2017, significantly more whiteflies were found in the control plots compared with diversified treatments and the M-Pede-treated plots (F=4.12; df=4, 200; p=0.0032; Figure 2a). Densities were highest on the fifth WAP (F = 7.34; df = 4, 200; p < 0.0001), but there were no interaction effects between treatment and time (F = 1.43; df = 4, 200; p = 0.1339).



FIGURE 2 Mean (+SE) number of adult whiteflies sampled in situ per bed (a) and on yellow sticky traps (YST) per bed (b) in 2016 and in 2017. Bars within the same year followed by different letters are significantly different ( $p \le 0.05$ ).

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#### 3.1.2 | Yellow sticky traps

In 2016, the M-Pede-treated plots had 54% and 59% fewer whiteflies than the mixed and control plots, respectively (F=3.70; df=4, 200; p=0.0065; Figure 2b). Differences were observed over time (F=58.83; df=4, 200; p<0.0001), but there were no interaction effects (F=0.81; df=16, 200; p=0.6732). In 2017, the cowpea and mixed plots had 68% and 51% fewer whiteflies than the control plots, respectively (F=4.10; df=4, 240; p=0.0032; Figure 2b). Densities differed over time (F=3.63; df=5, 240; p=0.0036) and between treatments and time (F=1.62; df=20; 240; p=0.0493).

#### 3.1.3 | Nymphal whiteflies

In 2016, there were significantly more nymphal whiteflies in the control than in the diversified treatments and the M-Pede-treated plots (F=3.80; df=4, 160; p=0.0058; Table 1). More nymphs were observed on the 5th WAP (F=6.08; df=3, 160; p=0.0006), but there was no interaction effect between treatment and time (F=1.26; df=12, 160; P=0.2481). In 2017, there were no differences among treatments (F=0.62; df=4, 100; p=0.6490; Table 1). More nymphs were observed on the fourth and sixth WAP (F=23.83; df=4, 100; p<0.0001), but there was no interaction effect (F=1.36; df=16, 100; p=0.1890).

#### 3.2 | Diseases and physiological disorders

Viral symptoms were not observed on the squash plants or on the fruit for either of the 2 years. In 2016, the SSL disorder rating was significantly lower in the cowpea plots compared with all other treatments (F = 4.52; df = 4, 600; p = 0.0013; Table 1). There was less damage on the fourth WAP than on any other week (F = 3.85; df = 4, 600; p = 0.0043) with an interaction between treatments and weeks (F = 3.56; df = 16, 600; p < 0.0001). In 2017, the control plants had a significantly higher SSL rating than the cowpea intercropped and M-Pede-treated plots (F = 3.98; df = 4, 600; p = 0.0034; Table 1). The fourth WAP had the fewest number of whiteflies (F = 27.82; df = 4, 600; p < 0.0001). There were also

interactions between treatments and time (F=3.11; df=16, 600; p < 0.0001).

## 3.3 | Aphids

Several aphid species were recorded in all the samples in the study for the fall of 2016 and 2017. The three aphids of concern sampled were the cowpea aphid (*Aphis craccivora* Koch), green peach aphid (*Myzus persicae* Glover) and melon aphid (*Aphis gossypii* Glover). Other wheat and grass aphids included *Rhopalosiphum nymphaeae* L., *Schizaphis* spp., *Lipaphis pseudobrassicae* Davis and *Tetraneura* sp. Hartig.

#### 3.3.1 | In situ counts

In 2016, the M-Pede treatment had 50% and 38% fewer aphids than the cowpea intercropped plots and the control plots, respectively (*F*=4.28; df=4, 200; *p*=0.0025; Figure 3a). Aphids were in the highest numbers three WAP and significantly decreased the following weeks (*F*=27.76; df=4, 200; *p*<0.0001). Marginal significant differences were observed between treatments and time (*F*=1.68; df=16, 200; *p*=0.05). Similarly, in 2017, M-Pede-treated plots had 60%, 52% and 40% fewer aphids than the cowpea, mixed and control plots, respectively (*F*=5.35; df=4, 200; *p*=0.0004; Figure 3a). The seventh WAP had the greatest number of aphids (*F*=23.72; df=4, 200; *p*<0.0001), but there were no interaction effects (*F*=0.70; df=4, 200; *p*=0.7868).

#### 3.3.2 | Pan traps

In 2016, the M-Pede-treated plots had 43% fewer aphids than the cowpea intercropped plots (F=2.55, df=4, 200; p=0.0404; Figure 3b). There were significant differences over time (F=3.80; df=4, 200; p=0.0055), but there were no interaction effects (F=0.83; df=16, 200; p=0.6470). In 2017, the marigold, mixed and M-Pede-treated plots had between 43% and 44% fewer aphids than the control (F=2.67; df=4, 200; p=0.0056; Figure 3b). Differences

Control	0.87±0.29 a	$1.65 \pm 0.15$	1.98±0.08 a	2.18±0.07 a
Note: Means in co	lumns followed by diffe	rent letters are sig	nificantly different	(p≤0.05). Reported
means and SE are	back-transformed.			

<sup>a</sup>M-Pede application rate: 59 mL/ha.

	Mean no. nymphal whiteflies/leaf		Mean SSL rating/bed	
Treatment	2016	2017	2016	2017
Marigold	$0.18 \pm 0.11 \text{ b}$	$2.00 \pm 0.16$	1.93±0.08 a	1.90±0.08 ab
Cowpea	0.17±0.09 b	$1.30 \pm 0.12$	$1.69 \pm 0.10$ b	1.95±0.09 b
Mixed	$0.21 \pm 0.10 \text{ b}$	$2.15\pm0.17$	$2.05 \pm 0.08$ a	$2.16\pm0.08$ ab
M-Pede <sup>a</sup>	$0.31\pm0.15$ ab	$2.05 \pm 0.10$	$1.86\pm0.09$ ab	1.85±0.08 b
Control	$0.87 \pm 0.29$ a	$1.65\pm0.15$	$1.98\pm0.08$ a	$2.18 \pm 0.07$ a

 TABLE 1
 Mean (±SE) number of

 nymphal whiteflies per leaf and squash

# silverleaf (SSL) index rating per bed for 2016 and 2017.

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were seen over time (F=50.06; df=4, 200; p < 0.0001), but there were no interaction effects (F = 1.28; df = 16, 200; p = 0.2346).

#### 3.4 **Parasitoids**

#### In situ counts 3.4.1

In 2016, the number of parasitoids observed in situ differed significantly by treatment, where the cowpea intercropped plots had 55% and 53% more parasitoids than the M-Pede-treated plots and the control, respectively (F=5.02; df=4, 200; p=0.0007; Table 2).



FIGURE 3 Mean (±SE) number of aphids sampled in situ per bed (a) and in pan traps per bed (b) in 2016 and 2017. Bars within the same year followed by different letters are significantly different (p ≤ 0.05).

TABLE 2 Mean (±SE) number of parasitoids for 2016 and 2017 found in situ and on yellow sticky traps (YST).

Highest densities were observed three and seven WAP (F = 22.35; df=4, 200; p < 0.0001), but there were no interaction effects (F=1.18; df=16, 200; p=0.2868). In 2017, the marigold and mixed intercropped treatments had significantly more parasitoids than the other three treatments and 60% greater numbers than the control (F=10.26; df=4, 200; p<0.0001; Table 2). The fourth and fifth WAP had the highest densities (F = 8.61; df = 4, 200; p < 0.0001), but there was no interaction effect between treatments and time (F=1.28; df=4, 200; p=0.2129).

#### 3.4.2 Yellow sticky traps

Hymenopteran parasitoids recovered from the yellow sticky traps were classified at the family level. They included Aphelinidae, Braconidae, Encyrtidae, Eulophidae, Ceraphronidae, Cynipidae, Dryinidae, Ichneumonidae, Mymaridae, Mymmaromatidae, Platygastridae, Pteromalidae, Signiphoridae and Trichogrammatidae. In 2016, there were 45% and 43% more parasitoids found in the marigold and cowpea intercropped plots than in the control, respectively (F=2.74; df=4, 200; p=0.0304; Table 2). The highest densities were seen four WAP (F = 23.66; df = 4, 200; p < 0.0001), but there were no interaction effects (F = 1.49; df = 16, 200; p = 0.1084). In 2017, there were no significant differences among treatments (F=0.74; df=4, 240; p = 0.5673; Table 2). The highest density of parasitoids was observed on the seventh WAP (F = 20.36; df = 5, 240; p < 0.0001), but there were no interaction effects (F = 1.17; df = 20, 240; p = 0.2796).

#### Aphelinidae

Aphelinids accounted for 17% and 12% of all parasitoids recovered from the sticky traps in 2016 and 2017, respectively. The highest number of aphelinids were present in the cowpea intercropped plots compared with the marigold intercropped plots (F=2.76; df=4, 200; p = 0.0292; Table 3). Densities were highest on the fourth WAP (F=15.24; df=4, 200; p < 0.0001) but there were no interaction effects (F = 1.54; df = 4, 200; p = 0.0917). In 2017, while squash intercropped with marigold had 26% of all aphelinids, treatments did not differ significantly among each other (F = 1.30; df = 4, 240;

	In situ counts		YST		
Treatment	2016	2017	2016	2017	
Marigold	$1.27\pm0.22$ ab	$1.05 \pm 0.17$ a	$10.35 \pm 0.78$ a	$6.45\pm0.61$	
Cowpea	$2.07 \pm 0.37$ a	0.80±0.20 b	$11.10 \pm 1.12$ a	$6.10\pm0.53$	
Mixed	$1.47\pm0.23~ab$	$1.07\pm0.16$ a	$8.57\pm0.83$ ab	$6.12 \pm 0.58$	
M-Pede <sup>a</sup>	$1.10\pm0.37$ b	$0.35 \pm 0.10 \text{ b}$	9.07±0.83 ab	$6.04 \pm 0.61$	
Control	$1.05 \pm 0.28$ b	$0.20\pm0.06$ b	8.30±0.96 b	$5.53 \pm 0.63$	

Note: Parasitoids were identified to family level for Aphelinidae, Braconidae, Encyrtidae,

Eulophidae, Ceraphronidae, Cynipidae, Dryinidae, Ichneumonidae, Mymaridae, Mymmaromatidae, Platygastridae, Pteromalidae, Signiphoridae and Trichogrammatidae.

Means in columns followed by different letters are significantly different ( $p \le 0.05$ ). Reported means and SE are back-transformed.

<sup>a</sup>M-Pede application rate: 59 mL per ha.

TABLE 3 Mean (±SE) number of Aphelinidae (including genera *Encarsia* spp. and *Eretmocerus* spp.) and braconidae found on yellow sticky traps (YST) for 2016 and 2017.

		Treatments				
Year	Family/Genus	Marigold	Cowpea	Mixed	M-Pede <sup>a</sup>	Control
2016	Aphelinidae	1.57±0.27 ab	2.67±0.50 a	1.27±0.21 b	$1.60\pm0.25$ ab	$1.32 \pm 0.20$ b
	Encarsia spp.	$2.25 \pm 0.55$	$1.87 \pm 0.47$	$1.25 \pm 0.25$	$1.12 \pm 0.39$	$1.25 \pm 0.31$
	Eretmocerus spp.	$0.25 \pm 0.16$	$0.62 \pm 0.18$	$0.25 \pm 0.25$	$0.62 \pm 0.26$	$0.25 \pm 0.15$
	Braconidae	2.17±0.35 b	3.60±0.52 a	$1.80\pm0.29$ b	$2.40\pm0.31$ ab	$2.32\pm0.30$ ab
2017	Aphelinidae	$0.94 \pm 0.18$	$0.58 \pm 0.13$	$0.64 \pm 0.17$	$0.91 \pm 0.20$	$0.64 \pm 0.14$
	Encarsia spp.	0.25±0.11 a	$0.12\pm0.08$ ab	$0.06 \pm 0.06$ ab	$0\pm0$ ab	0±0 b
	Eretmocerus spp.	0.43±0.15 a	$0.31\pm0.11$ ab	$0.06\pm0.06$ ab	$0.06 \pm 0.06$ ab	$0.05 \pm 0.05$ b
	Braconidae	$0.45 \pm 0.12$	$0.68 \pm 0.11$	$0.68 \pm 0.20$	$0.54 \pm 0.10$	$0.51 \pm 0.10$

Note: Means in rows followed by different letters are significantly different (p ≤ 0.05). Reported means and SE are back-transformed. <sup>a</sup>M-Pede application rate: 59 mL per ha.

p=0.2723; Table 3). There were differences across time with the seventh WAP showing the highest number of aphelinids (F=12.03; df=5, 240; p<0.0001) as well as interaction effects (F=2.09; df=20, 240; p=0.0054). Two key genera recovered from the yellow sticky traps included *Encarsia* spp. and *Eretmocerus* spp. In 2016, *Encarsia* spp. (F=1.21; df=4, 200; p=0.3238; Table 3) and *Eretmocerus* spp. (F=1.30; df=4, 200; p=0.2923; Table 3) were not found in significant differences among any of the treatments. In 2017, both genera (*Encarsia* spp. [F=2.63; df=4, 240; p=0.0409; Table 3] and *Eretmocerus* spp. [F=3.39; df=4, 240; p=0.0130; Table 3]) were found in greater numbers in the squash intercropped with marigold than in the control.

#### Braconidae

Braconids accounted for 5% and 8% of all parasitoids recovered from the yellow sticky traps, in 2016 and 2017, respectively. In 2016, there were 45% more braconids found in the cowpea intercropped plots than any of the other treatments (F=3.42; df=4, 200; p=0.0100; Table 3). Differences were observed by week (F=5.43; df=4, 200; p=0.0004), but there was no interaction effect (F=0.55; df=16; 200; p=0.9173). In 2017, the squash intercropped with cowpea and the mixed treatment hosted 26% and 28% of braconids, respectively, but there were no significant differences among treatments (F=1.32; df=4, 240; p=0.2623; Table 3). The highest density of braconids was seen on the seventh WAP (F=2.77; df=5, 240; p=0.0192), but there were no interaction effects (F=1.33; df=20, 240; p=0.1653).

#### 3.5 | Predators

Insect predators were identified at the family level and included minute pirate bugs (Anthocoridae), ground beetles (Carabidae), big-eyed bugs (Geocoridae), lacewings (Chrysopidae), lady beetles (Coccinellidae), long-legged flies (Dolichopodidae), rove beetles (Staphylinidae) and hover flies (Syrphidae). Coccinellidae, Syrphidae and Anthocoridae were identified to genera or species levels. Four species of lady beetles were identified, including *Delphastus catalinae* (Horn), *Coccinella septempunctata* L., *Cycloneda sanguinea* L. and *Chilocorus* sp. Leach. Four genera of Syrphids were identified, including *Melangyna* sp. Verrall, *Allograpta* sp. Osten Sacken, *Toxomerus* sp. Macquart and *Dioprosopa* sp. Hull. Anthocoridae was identified as *Orius* sp. Wolff. Spiders were identified to the order level (Araneae).

#### 3.5.1 | In situ counts

In 2016, predator densities were not significantly greater in any of the treatments (F=1.01; df=4, 200; p=0.4046; Table 4) and their numbers significantly increased between the third and four WAP (F=7.57; df=4; 200; p < 0.0001). There were no interaction effects (F=0.73; df=16, 200; p=0.7624). Adult and immature coccinellids accounted for 41% of the insect predator families recovered. Coccinellids were the only family showing significant differences among treatments, with the highest densities in the cowpea treatments compared with the M-Pede and the control plots (F=2.74; df=4, 200; p=0.0305; data not included in table). The fourth WAP had the highest numbers of coccinellids (F = 2.74; df = 4, 200; p = 0.0305), but there were no interaction effects (F = 0.64; df = 16, 200; *p*=0.8436). In 2017, the mixed treatment had 180% and 62% more predators than the M-Pede-treated and control plots, respectively (F=4.20; df=4, 200; p=0.0029). Additionally, all diversified plots hosted significantly more predators than the control plots (Table 4). Predators were consistently present throughout the study with no differences across weeks (F=0.76; df=4, 200; p=0.5535) or interaction between treatment and time (F = 0.3703; df = 16, 200: p = 0.3703). Araneae accounted for 65% of all predators recovered and were the only predator found in significantly greater numbers in the marigold and mixed treatments compared with the M-Pedetreated plots (F=4.18; df=4, 200; p=0.0029; data not included in TABLE 4 Mean ( $\pm$ SE) number of predators found in situ and in the pan traps.

	In situ counts		Pan traps	
Treatment	2016	2017	2016	2017
Marigold	$0.42 \pm 0.12$	$0.37\pm0.10$ ab	$2.52 \pm 0.16$ a	$2.75\pm0.46$ ab
Cowpea	$0.72 \pm 0.17$	$0.35\pm0.09$ ab	$1.65\pm0.12$ ab	$1.55\pm0.30$ b
Mixed	$0.55 \pm 0.12$	$0.57 \pm 0.12$ a	$2.17\pm0.14$ ab	$2.60\pm0.41$ ab
M-Pede <sup>a</sup>	$0.37 \pm 0.09$	0.05±0.03 b	$0.95 \pm 0.11 \text{ b}$	4.40±0.55 a
Control	$0.45 \pm 0.12$	$0.30\pm0.10~\text{ab}$	$0.77 \pm 0.09 \text{ b}$	$3.25\pm0.48$ ab

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Note: Predators included Araneae, Anthocoridae, Carabidae, Coccinellidae, Chrysopidae (in situ only), Dolichopodidae, Geocoridae, Staphylinidae and Syrphidae (pan trap only).

Means in columns followed by the same letter(s) are not significantly different ( $p \le 0.05$ ). Reported means and SE are back-transformed.

<sup>a</sup>M-Pede application rate: 59 mL per ha.

table). There was no significance among weeks (F=0.11; df=4, 200; p=0.9787) or interaction effects (F=0.76; df=16, 200; p=0.7315).

#### 3.5.2 | Pan traps

In 2016, 81% and 68% more predators were present in the cowpea intercropped plots than in the M-Pede-treated plots and the control plots, respectively (F=3.79; df=4, 200; p=0.0065; Table 4). The highest number of predators were recorded four WAP (F = 5.35; df = 4, 200; p = 0.0006), but there was no interaction effect (F = 1.19; df = 16, 200; p = 0.2870). Dolichopodidae were found in significantly higher quantities in cowpea compared with the M-Pede-treated plots and the control plots (data not included in table: F = 4.59; df = 4, 200; p = 0.0015). Dolichopodidae differed by weeks (F = 4.52; df = 4, 200; p = 0.0017), but there were no interaction effects (F = 1.28; df = 16, 200; p = 0.2169). In 2017, M-Pede-treated plots had 46%, 96% and 52% more predators than marigold, cowpea and mixed intercropped plots, respectively (F = 3.88; df = 4, 200; p = 0.0064; Table 4). Predators were consistently present throughout the study (F=0.22; df=4, 100; p = 0.9246) but there were no interaction effects (F = 0.92; df = 16, 100; p = 0.5550). No specific predator family was found in significantly higher numbers among any of the treatments.

#### 3.6 | Marketable yields

Overall, in 2016, plots with intercropped African marigolds yielded 58% and 71% more marketable squash than the control and the M-Pede-treated plots, respectively. The cowpea and mixed intercropped plots were also significantly higher than both the control and M-Pede-treated plots (F=15.18; df=4, 200; p<0.0001; Figure 4). The ninth WAP saw the highest yields (F=40.90; df=4, 200; p<0.0001) as well as interaction effects (F=1.89; df=20, 200; p=0.0240). In 2017, the mixed intercropped plots yielded 19% more squash than the control plots (F=2.30; df=4, 360; p=0.05; Figure 4). The highest yields were observed eight WAP (F=70.33; df=4, 200;



**FIGURE 4** Mean marketable squash yield (kg) per bed for the fall of 2016 and 2017. Bars within the same year followed by different letters are significantly different ( $p \le 0.05$ ).

p < 0.0001), but there were no interaction effects (F=0.61; df=12, 360; p=0.8346).

## 4 | DISCUSSION

Organic squash growers have limited options when managing arthropod pests. This study investigated complementary field production practices, including intercropping and the application of M-Pede insecticidal soap to manage the silverleaf whitefly and aphid pests of zucchini squash. Additionally, we assessed these treatments on the natural enemy population and marketable squash yields.

We found that the diversified cropping treatments as well as the M-Pede were the most successful at reducing nymphal whitefly numbers. Zavaleta-Mejia and Gomez (1995) found lower nymphal whitefly populations and lower incidence of virus symptoms on tomato plants in experiments investigating the effects of intercropping marigolds and varying planting dates on tomato pests. The management of nymphal whiteflies is important because they induce the SSL disorder when feeding on the phloem sap with their needle-like mouthparts and leave openings where air flows into the layers of the leaves, causing the silvered appearance (Costa et al., 1993; Jimenez et al., 1994). The lowest SSL disorder ratings were recorded in the

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cowpea intercropped plots. These plots harboured high numbers of parasitoids and predators in the families Aphelinidae (*Encarsia* spp. and *Eretmocerus* spp.), Coccinellidae and Dolichopopidae. It is possible that these beneficial insects may have provided some biological control services against whitefly nymphs, as shown in previous studies (Gerling et al., 2001; Gorri et al., 2015; Lucas et al., 2004; Razze et al., 2016; Sadhana et al., 2022; Tan et al., 2016; Togni et al., 2018). Treatment effects on whitefly nymphs were not observed in 2017 when the whitefly population was higher. It is not clear why the differences between the 2 years occurred, but field conditions due to a hurricane in 2017 may have affected whitefly population and other plant growth parameters. This was evident since the intercropped plants grown in 2017 were generally smaller with higher populations of whiteflies.

Cowpea, the mixed planting of cowpea and marigold, and M-Pede provided the most consistent suppression of whitefly adult populations. The potential of M-Pede for whitefly management in organic systems has been demonstrated under greenhouse conditions (Razze et al., 2016), but few studies have shown its effectiveness under organic field conditions.

M-Pede also provided the most reliable suppression of aphids, which concurs with Dively et al. (2020), where M-Pede reduced green peach aphids by 42% in greenhouse settings. The mechanism involves desiccating the insect cuticle, obstructing spiracles and causing asphyxia (Henn & Weinzierl, 1989). We did not find any deleterious effect of M-Pede on the natural enemy populations. In greenhouse and laboratory studies, M-Pede was also found to be non-toxic to *D. catalinae* when adults were released 5 days post-application and when applied directly on *C. sanguinea* (Hall & Richardson, 2012; Razze et al., 2016).

Despite high adult whitefly numbers in 2016, squash plants did not exhibit viral symptoms on the leaves or fruits for either year. Viral symptoms may have been absent because of the lower-thanusual temperatures during that fall growing season, which may have affected the whiteflies' efficiency of transmitting viruses. It takes between 3 and 4 weeks for plants to begin exhibiting signs of viruses (Gordon, 2014), and in 2016, the population of nymphs was low, averaging less than 0.9 nymphs per leaf and less than 10 adults per leaf. Adult and nymphal whiteflies peaked in the third and fifth WAP, respectively. This meant that viral symptoms would have begun showing in the seventh WAP when squash season was ending. In 2017, observations began in November, and there were low adult whitefly densities from the beginning to the end of the squash season, with the population rarely exceeding about 2.15 whiteflies per leaf. Leaf crumpling and distortion were only observed in the cowpea plants and were heavier in the fall of 2016. While the cowpea aphid and the melon aphid both feeds heavily on legumes and transmit zucchini yellow mosaic virus (ZYMV), the zucchini squash variety used in our study, 'Cashflow', is resistant to ZYMV. This may indicate why viral symptoms were not observed.

In both years, relatively high densities of aphids were recovered from the cowpea intercropped plots. Aphids can reproduce parthenogenically all year round in Florida; thus, it is not surprising that

aphids were present from September to November. Our findings confer with Lopez and Liburd (2022), showing a higher aphid population when squash was planted next to cowpea. The high density of aphids justifies the higher presence of coccinellids in the cowpea, including D. catalinae, C. septempunctata, Cy. sanguinea and Chilocorus sp. These coccinellids have been shown to be effective suppressants of aphids (Bista & Omkar., 2013; Isikber, 2005; Khan & Zaki, 2007; Razze et al., 2016; Sharma et al., 1991). Coccinellids have also been observed in high numbers when cabbage and squash were intercropped with cowpea (Borkakati et al., 2019; Lopez & Liburd, 2022). Through in situ counts and YST, we show that marigold and cowpea plots supported a higher density of parasitoids than the control for both years. Encarsia spp. and Eretmocerus spp., two of the silverleaf whitefly's key parasitoids (Liu et al., 2014), were found in significantly and numerically high numbers on marigold plots for 2016. This is corroborated by research by Heinz and Parella (1990), where Encarsia formosa Gahan and green lacewings suppressed the greenhouse whitefly Trialeurodes vaporariorum Westwood, A.gossypii, and M. persicae, to manageable levels. High numbers of parasitoids, including braconidae, were observed in the cowpea treatment in 2016. While braconids did not entirely suppress aphids, they may have kept aphids from rising to damaging levels. Similarly, Gamal et al. (2022) found four braconid species attacking cowpea aphids in faba beans.

The flowers in African marigolds and legumes can provide predators with essential sources of energy (Koptur, 1992). In 2017, squash in the mixed treatment had the greatest densities of predators found in situ, which consisted mainly of Araneae. Assemblages of spiders can greatly benefit farmers by controlling pests and reducing their economic losses (Welch et al., 2016). In our study, while spiders may not have entirely suppressed aphids, they likely assisted in regulating their populations.

We obtained superior yields in diversified treatments compared with the control. In the first year of our study, intercropping squash with marigold increased marketable yields by 58%; with cowpea by 43%; and with the mixed treatment by 37% compared with the control plots. Previous studies have also shown significantly higher squash yields when grown with and after legumes (Peoples et al., 1995; Sant'Anna et al., 2018) and when grown alongside marigolds (Lopez & Liburd, 2022). A higher number of predators and parasitoids found in the diversified treatments may have indirectly influenced the higher proportion of marketable squash yields through their biological control services on nymphal whiteflies. In 2017, we observed moderately higher marketable squash yields than in 2016. It is likely that environmental factors (hurricane Irma preceding the experiment in 2017 and cold stress) may have affected arthropod numbers in the system. In addition, while M-Pede reduced pest pressure, this was not reflected in the marketable yield for that year. This may have been due to the wider distribution of predators among all the treatments for the second year.

We conclude that the effectiveness of marigolds, cowpea and mixed plantings are supported by our results—when intercropped with zucchini squash, there is an enhanced establishment of beneficial arthropods and potential biological control services, which may also result in increased yields. These findings are important to an organic cucurbit grower wishing to increase profitability of their cash crop while diversifying their production system. In following an IPM program, we encourage that M-Pede be used as a last resort when cultural and biological control tactics are not effective at reducing pest numbers below the economic threshold. Future research prospects in using these IPM practices to enhance squash marketability for growers include investigating their suppressive effects on other pests including lepidopteran herbivores and plant parasitic nematodes. In addition, more research is needed on the dualistic effects of African marigolds and cowpea on improving soil structure and microbial community composition in organic cucurbit systems.

#### AUTHOR CONTRIBUTIONS

**Marice Lopez:** Conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, writing—original draft; **Oscar Liburd:** conceptualization, funding acquisition, project administration, resources, supervision, validation, visualization, writing—review and editing.

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#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest with this work.

#### DATA AVAILABILITY STATEMENT

The data that support the finding of this study are available at figshare https://doi.org/10.6084/m9.figshare.22714480.v1.

#### ORCID

Marice Lopez https://orcid.org/0000-0003-2992-9975 Oscar E. Liburd https://orcid.org/0000-0001-8827-5823

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#### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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