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Effects of biological insecticides on the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), in sorghum

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ABSTRACT

Melanaphis sacchari (Zehntner) is a major pest of sorghum, *Sorghum bicolor* (L.) Moench, in the United States and neighboring Caribbean countries including Haiti. Laboratory, greenhouse, and field experiments were conducted to determine the effects of biological insecticides on *M. sacchari* infesting sorghum. Azadirachtin, pyrethrins, *Beauveria bassiana* strain GHA, *Isaria fumosorosea* Apopka strain 97, *Chromobacterium subtsugae* strain PRAA4-1^T, *Burkholderia* spp. strain A396, and vetiver oil were compared to a conventional insecticide, flupyradifurone. In the laboratory, sorghum leaf discs were sprayed with treatment solutions and subsequently infested with *M. sacchari* nymphs. In the greenhouse, potted sorghum plants were sprayed with treatment solutions before or after infestation with *M. sacchari* nymphs. In the field, plots exposed to natural *M. sacchari* infestations were sprayed with treatment solutions. All insecticide treatments except *I. fumosorosea* and *Burkholderia* spp. were associated with 58–100% aphid mortality after 72 h in the laboratory, which was greater than the 20% mortality observed in the non-treated control. In the greenhouse, azadirachtin and pyrethrins were the biological insecticides associated with the lowest aphid infestation levels 7 days after treatment. Flupyradifurone was associated with the greatest mortality in the laboratory and the lowest infestation levels in the greenhouse. In the field, decreases in aphid infestation levels relative to the non-treated control were not observed although flupyradifurone was consistently associated with the lowest infestations. Our results suggest that biological insecticides including azadirachtin, pyrethrins, and *B. bassiana* could potentially control *M. sacchari* infestations in sorghum if applied under favorable environmental conditions.

1. Introduction

Melanaphis sacchari (Zehntner), also known as the sugarcane aphid, has historically been a serious insect pest of sorghum, *Sorghum bicolor* (L.) Moench, in Africa and Asia (Singh et al., 2004). This insect has become a major pest in sorghum in the United States since 2013 (Villanueva et al., 2014; Bowling et al., 2016) and outbreaks threatening sorghum production in neighboring Caribbean countries including Haiti have occurred since 2015 (Gabriel, 2016). *Melanaphis sacchari* nymphs and adults suck plant sap and excrete honeydew that favors the growth of black sooty mold, a fungus that covers leaves and can cause chlorosis because of lack of photosynthesis (Singh et al., 2004). In North America, *M. sacchari* infestations can cause grain sorghum yield losses approaching 100% (Brewer et al., 2017). Infestations can also impede combine harvester performance and prevent grain separation from leaves and stalks, which leads to significant grain losses. In addition, *M. sacchari* can impact forage sorghum harvest by affecting the cutting and baling efficiency and decreasing forage quality due to mold (Villanueva et al., 2014; Bowling et al., 2016).

Melanaphis sacchari management in sorghum in the United States has primarily relied on the use of resistant varieties and conventional synthetic insecticides including neonicotinoid seed treatments and foliar applications of sulfoxaflor and flupyradifurone (Villanueva et al., 2014; Brown et al., 2015; Knutson et al., 2015; Brewer et al., 2016). However, the frequent use of insecticides targeting the nicotinic acetylcholine receptor might contribute to the development of insecticide resistant

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aphid populations (Bowling et al., 2016; Szczepaniec, 2018; Etheridge et al., 2019; IRAC (Insecticide Resistance Action Committee), 2020). In addition, the use of these insecticides in other cropping systems has been associated with concerns for non-target arthropods, including pollinators (Zhu et al., 2015; Krupke et al., 2017; Tsvetkov et al., 2017). Insecticides of biological origin, hereafter referred to as biological insecticides, have a diversity of modes of action allowing rotation to mitigate the development of insecticide resistance (Copping and Menn, 2000; Chandler et al., 2011). In addition, these biological insecticides generally have favorable ecotoxicological profiles relative to conventional synthetic insecticides (Copping and Menn, 2000; Bahlai et al., 2010; Marrone, 2019), and their adoption may offer additional reduced-risk production practices in developing countries where farmers are not well equipped and trained to use pesticides (USAID, 2010). Thus, biological insecticides may be a suitable tactic to be integrated into a management program for *M. sacchari* in the United States and in developing countries such as Haiti.

Biological insecticides based on botanical extracts, entomopathogenic fungi, or entomopathogenic bacteria and their toxins have adverse effects against insect pests including aphids (Stark and Walter, 1995; Selvaraj and Kaushik, 2014; Kuhar and Doughty, 2016). For instance, insecticides based on neem, *Azadirachta indica* A. Juss., seed extracts can be effective aphicides (Lowery and Isman, 1994; Krais and Cullen, 2008). Neem seed oil and its main insecticidal component, azadirachtin, caused nymphal mortality, prolonged developmental time, and reduced fecundity of the soybean aphid, *Aphis glycines* Matsumura (Kraiss and Cullen, 2008). A mixture of neem seed oil and azadirachtin reduced the life span and fecundity of the pea aphid, *Acyrthosiphon pisum* (Harris), and was lethal by affecting the molting process (Stark and Walter, 1995). For *M. sacchari*, azadirachtin caused negative effects under laboratory conditions (Yadav et al., 2016) but was associated with inconsistent control in the field (Buntin and Roberts, 2016; Díaz-Nájera et al., 2018).

Pyrethrins, a mixture of six active compounds extracted from *Chrysanthemum cinerariifolium* (Trevir.) Vis. plants, control insect pests including aphids (Casida, 1980; Khater, 2012; Singh, 2014). Field studies showed that pyrethrins controlled *A. pisum* and the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze), in Bulgaria (Niklova, 2016). In addition, pyrethrins negatively affected the brown marmorated stink bug, *Halyomorpha halys* (Stål), in laboratory bioassays (Lee et al., 2014; Morehead and Kuhar, 2017).

Oil extracted from vetiver grass, *Chrysopogon zizanioides* (L.) Roberty, is composed of α-vetivones, β-vetivones, khusinol, khusilal, diethyl phthalate, vetiselinol, khusimol, isovelencenol, and vetivenic acid. This botanical extract has been reported as an effective repellent and toxicant of insect pests including the red imported fire ant, *Solenopsis invicta* Buren, German cockroach, *Blattella germanica* (L.), and Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Zhu et al., 2003; Henderson et al., 2005a, 2005b). In addition, vetiver grass extracts caused more than 50% mortality in the cowpea weevil, *Callosobruchus maculatus* (F.), under laboratory conditions (Pangnakorn, 2009).

Beauveria bassiana (Bals. -Criv.) Vuill. is an entomopathogenic fungus that was found naturally infecting *M. sacchari* in several regions in Mexico (Zambrano-Gutierrez et al., 2019). In laboratory studies, *B. bassiana* strain ABNB6 attacked *M. sacchari* (Harris-Shultz et al., 2020), and *B. bassiana* strain CKB-48 caused more than 90% nymphal mortality in *M. sacchari* and six other aphid pest species (Maketon et al., 2013). In another laboratory study, *B. bassiana* negatively affected several aphid species including the greenbug, *Schizaphis graminum* (Rondani), and the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Akmal et al., 2013). In addition, greenhouse and field experiments showed that *B. bassiana* strain HaBa controlled the cowpea aphid, *Aphis craccivora* Koch (Selvaraj and Kaushik, 2014). Another fungus, *Isaria fumosorosea* (Wize) Brown & Smith, provided as much as 100% control of the brown citrus aphid, *Toxoptera citricidus* (Kirkaldy) (Hunter et al., 2011). *Isaria fumosorosea* also had insecticidal effects against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), by causing as much as 100% egg and larval mortality (Sabbour and Abdel-Raheem, 2014). Gandarilla-Pacheco et al. (2015) reported that *I. fumosorosea* caused 40 and 57% mortality in neonate beet armyworm, *Spodoptera exigua* (Hübner), and corn earworm, *Helicoverpa zea* (Boddie), respectively. However, field evaluations of *B. bassiana* strains ABNB6 and GHA, and of *I. fumosorosea* Apopka strain 97, did not decrease *M. sacchari* infestation levels in grain sorghum (Harris-Shultz et al., 2020).

The bacteria *Chromobacterium subtsugae* strain PRAA4-1^T inhibited feeding or caused mortality in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), the sweet potato whitefly, *Bemisia tabaci* (Gennadius), and seven other insect pest species in laboratory bioassays (Martin et al., 2007). *Chromobacterium subtsugae* also had adverse effects on the melon aphid, *Aphis gossypii* Glover, and the black pecan aphid, *Melanocallis caryaefoliae* (Davis) (Shapiro-Ilan et al., 2013; Kuhar and Doughty, 2016). Other bacteria, *Burkholderia* spp. strain A396, showed suitable control of the cranberry fruitworm, *Acrobasis vaccinii* Riley, in blueberry, *Vaccinium* spp. (Wise et al., 2015). In addition, laboratory bioassays showed that *Burkholderia* spp. strain A396 caused as much as 85% *S. exigua* mortality (Cordova-Kreylos et al., 2013).

Azadirachtin, pyrethrins, *B. bassiana*, *I. fumosorosea*, *C. subtsugae*, and *Burkholderia* spp. are commercially formulated as biological insecticides in the United States. In addition, Haiti is the largest producer of vetiver in the world (Belhassen et al., 2015). Thus, commercial biological insecticides and vetiver oil may represent additional tactics to consider in an integrated pest management strategy for *M. sacchari* in sorghum in the United States and Caribbean countries such as Haiti. In this study, the efficacy of biological insecticides registered on numerous crops in the United States, as well as vetiver oil of Haitian origin, was evaluated in the laboratory, greenhouse, and field for control of *M. sacchari*.

2. Materials and methods

2.1. Experimental treatments

All experimental treatments (Table 1) were mixed in deionized water 30–60 min before use. Concentrations for the commercial biological insecticides were consistent with the highest registered field rates applied at a volume of application of 187 L/ha whereas the concentration for vetiver essential oil was 2% (v/v). The vetiver oil solution was warmed at 38 °C for 30 min and vigorously shaken for 30 s immediately before use to mix the oil in water. The concentration of flupyradifurone, which served as a conventional insecticide standard, was consistent with the highest field rate recommended by the manufacturer and applied at a volume of application of 187 L/ha.

2.2. Aphid colony

A colony of *M. sacchari* was initiated using a single apterous aphid collected on May 28, 2018 from a field of sweet sorghum (M-81E variety, Broadhead et al., 1981) at the University of Florida Institute of Food and Agricultural Sciences Everglades Research and Education Center (UF/IFAS EREC) in Belle Glade, FL. The aphids were reared on 2-4-week-old M-81E sorghum plants maintained in plastic pots (20.3-cm top diameter, 14.2-cm-deep) filled with potting soil (Miracle-Gro All Purpose Potting Mix, Scotts Miracle-Gro Company, Marysville, OH) at a density of one plant per pot. The plants were kept in a pop-up rearing cage (61 × 61 × 142 cm, Bioquip, Rancho Dominguez, CA) in a greenhouse. Once a week, eight plants were infested with 15 adults using a fine paintbrush. As needed, as many as 15 additional sorghum plants were infested with 15 adults per plant 1 day before laboratory and greenhouse experiments to produce 1-day-old nymphs for infestation in those experiments. Each adult produced an average of four nymphs in 1 day.

Table 1
Insecticide treatments evaluated on *Melanaphis sacchari* in laboratory, greenhouse, and field experiments, Belle Glade, FL, 2018–2019.

Treatment	Trade name	Manufacturer	Treatment formulation and active ingredient concentration	Formulation concentration in deionized water	Equivalent field rate (g a.i./ha)
Azadirachtin	Molt-X® EC	BioWorks, Victor, NY	Emulsifiable concentrate, 33.8 g a.i./L	3.9 ml/L	24.7
Pyrethrins	Pyganic® 5.0 EC	Valent U.S.A, Walnut Creek, CA	Emulsifiable concentrate, 49.1 g a.i./L	6.6 ml/L	61.0
Vetiver essential oil (Haitian origin)	–	Floracopeia, Grass Valley, CA	Oil, 100% a.i.	20.0 ml/L	3.7[a]
Beauveria bassiana strain GHA	BotaniGard® ES	BioWorks, Victor, NY	Emulsifiable suspension, 101.0 g a.i./L	12.5 ml/L	236.2
Beauveria bassiana strain GHA + pyrethrins	BotaniGard® Maxx	BioWorks, Victor, NY	Emulsifiable dispersible oil, 0.5 + 6.6 g a.i./L	12.5 ml/L	1.1 + 15.4
Isaria fumosorosea Apopka strain 97	PFR-97™ 20% WDG	Certis USA, Columbia, MD	Water dispersible granules, 200 g a.i./kg	12.0 g/L	448.3
Chromobacterium subtsugae strain PRAA4-1^T	Grandevo® WDG	Marrone Bio Innovations, Davis, CA	Water dispersible granules, 300 g a.i./kg	18.0 g/L	1008.8
Burkholderia spp. strain A396	Venerate® XC	Marrone Bio Innovations, Davis, CA	Water-based liquid concentrate, a.i. concentration not available	50.0 ml/L	9.4[a]
Flupyradifurone	Sivanto™ prime	Bayer CropScience, Research Triangle Park, NC	Soluble liquid, 200 g a.i./L	2.7 ml/L	102.3

[a] Liters of formulation/ha.

2.3. Laboratory experiment

An experiment was conducted in July 2018 at the UF/IFAS EREC to evaluate the effects of the seven commercial biological insecticides, vetiver oil, and flupyradifurone on *M. sacchari* mortality under laboratory conditions. Sorghum leaves collected from the upper canopy of plants in a 12-week-old field of M-81E were used in the laboratory to prepare leaf discs 7.5 cm in diameter. Although the field sustained an infestation of *M. sacchari* between 1 and 4 weeks after planting, plants were free of aphids at the time of leaf collection.

The nine insecticide treatments, as well as a non-treated control consisting of deionized water, were applied to individual leaf discs using a 60-ml amber glass bottle with mist sprayer (Katzco, Monroe, NY). Each side of a leaf disc received two sprays. The sprayer delivered 0.15 ml of solution for each spray on average. Thus, each leaf disc received an average of 0.60 ml of solution, a volume equivalent to that of one young sorghum plant being treated with 187 L/ha of broadcast spray solution in a field with 77,846 plants/ha covering 25% of the soil surface. Each leaf disc, which contained the midrib, was placed abaxial surface up in a 9-cm plastic Petri dish on top of a layer of filter paper saturated with deionized water. The upper surface of each leaf disc was allowed to air dry for 15–30 min before aphid infestation.

Five 1-day-old nymphs from the *M. sacchari* colony were placed at the center of each leaf disc using a fine paintbrush. Aphid mortality was determined 6, 24, 48, and 72 h following infestation. An aphid showing no perceptible movement after being prodded for 2–3 s with a fine paintbrush was considered dead. Three bioassays were conducted, each on a different date using a different aphid cohort and a freshly made set of insecticide solutions. Each bioassay included two-four replicates, for a total of ten replicates. Each replicate consisted of ten Petri dishes, one for each treatment and the non-treated control, and was placed on an individual wire shelf in a climate-controlled room at 26 °C, 40–60% RH, 12:12 (L:D) h.

2.4. Greenhouse experiments

Two experiments were conducted in July and August 2018 at the UF/IFAS EREC to evaluate the effects of the seven commercial biological insecticides, vetiver oil, and flupyradifurone on *M. sacchari* infestations developing on sorghum plants under greenhouse conditions. In the first experiment, the effects of treatments applied to sorghum plants already infested with aphids were evaluated whereas the effects of treatments applied to sorghum plants prior to aphid infestation were evaluated in a second experiment. In the two experiments, potted 2-week-old sorghum plants were used (see Aphid Colony section) and each plant was infested with five 1-day-old nymphs on the topmost leaf with a visible collar using a fine paintbrush. The nine insecticide treatments, as well as a non-treated control consisting of deionized water, were applied to individual plants using mist sprayers consistent with the laboratory experiment. Each plant received two sprays on each of two opposite sides, with one spray oriented upward and the second spray oriented downward for each side. Thus, each plant received an average of 0.60 ml of solution. Each experiment included five bioassays (replicates), each conducted on a different date using a different cohort of sorghum plants and aphids, as well as different insecticide solutions.

In each bioassay of the first experiment, each insecticide treatment or the non-treated control was sprayed on three plants infested with aphids 15–30 min earlier. The three plants for each treatment were immediately placed together in a screened tent-like cage (51 × 51 × 51 cm, Bug Dorm 2, Bioquip, Rancho Dominguez, CA). Subsequently, the ten cages containing three plants each were placed in a random order on a bench in the greenhouse. In each bioassay of the second experiment, a comparable method was used; however, sprayed plants were allowed to dry for 15–30 min before being infested with aphids and placed in cages. After 7 days, all plants of a bioassay were removed from the cages for whole-plant aphid counts. Temperature and relative humidity in the greenhouse were recorded every 15 min throughout the duration of the two experiments using a HOBO Pro V2 data logger (Onset Computer Corporation, Bourne, MA). Temperature averaged 25.9 °C (range: 21.9 °C–31.8 °C) and relative humidity averaged 95.7% (range: 57.4%–100.0%). The greenhouse used natural light.

2.5. Field experiments

Three experiments were conducted at the UF/IFAS EREC during spring 2018, fall 2018, and spring 2019 to further evaluate the effects of the seven commercial biological insecticides and flupyradifurone on *M. sacchari* infestations under field conditions. Sorghum fields were planted on April 9, 2018, October 9, 2018, and April 16, 2019 with variety M-81E at a density of 77,846 seeds/ha on 76.2-cm center using a four-row vacuum planter (John Deere Max Emerge, John Deere, Moline, IL). Seven insecticide treatments (spring and fall 2018) and eight insecticide treatments (spring 2019), as well as a non-sprayed control, were evaluated in a randomized complete block design with four blocks (replicates). Treatments were assigned to plots four rows wide and 10 m long.

Insecticide treatments were applied using a CO_2-pressurized backpack sprayer (R&D Sprayers, Opelousas, LA) with a two-row boom equipped with four TeeJet XR 8002VS nozzles (TeeJet Technologies, Wheaton, IL) spaced 38 cm apart and calibrated to deliver 187 L/ha at 207 kPa. Applications were initiated at first sign of *M. sacchari* infestations when plants exhibited three leaves with a visible collar, were at the early boot stage, and exhibited seven leaves with a visible collar in spring 2018, fall 2018, and spring 2019, respectively. In the spring 2018 experiment, insecticides were applied twice, 5 days apart, whereas in the two subsequent experiments insecticides were applied four times, 2–5 days apart over 2 weeks.

Aphid counts were taken from ten plants randomly selected on the two center rows of each plot in each experiment. In the spring 2018 experiment, whole-plant aphid numbers were determined. In addition to a pre-treatment count 1 day before the first insecticide application, one aphid count was taken 6 days after the first application. The experiment was subsequently terminated because excessive rainfall prevented additional insecticide applications and the aphid population declined considerably. In the fall 2018 and spring 2019 experiments, aphid numbers were determined for each plant on two leaves, one in the lower canopy and one in the upper canopy. The first leaf from the base of a plant with >75% of its surface green was considered as the lower leaf and the newest emerged leaf with a collar or the flag leaf, if present, was considered as the upper leaf. In addition to a pre-treatment count 1 day before the first insecticide application, aphid counts were taken weekly on three dates starting 7 days (fall 2018) or 6 days (spring 2019) after the first insecticide application. Temperature, relative humidity, and rainfall data recorded every 15 min between the first application and the last data collection were obtained from the Florida Automated Weather Network weather station located at the UF/IFAS EREC (fawn.ifas.ufl.edu). In spring 2018, temperature and relative humidity averaged 24.3 °C (range: 17.7 °C–32.2 °C) and 79.2% (range: 44.0%–100.0%), respectively. There were 2 days with ≥1 mm of rainfall over a 7-day period for a total of 8 mm. In fall 2018, temperature and relative humidity averaged 19.8 °C (range: 1.2 °C–31.7 °C) and 82.0% (range: 38.0%–100.0%), respectively. There was 1 day with 1 mm of rainfall over a 22-day period. In spring 2019, temperature and relative humidity averaged 26.3 °C (range: 17.6 °C–35.6 °C) and 84.3% (range: 33.0%–100.0%), respectively. There were 7 days with ≥1 mm of rainfall over a 21-day period for a total of 77 mm.

2.6. Statistical analyses

Data from all experiments were analyzed using linear mixed models (PROC GLIMMIX, SAS Institute Inc., 2016). For the laboratory experiment, aphid mortality expressed as the percentage of dead aphids was compared using a model with treatment, observation time, and their two-way interaction as fixed effects and bioassay, replicate(bioassay), and treatment × replicate(bioassay) as random effects. Thus, a variance component covariance structure was used to account for the effect of repeated measures. For each greenhouse experiment, whole-plant aphid numbers were compared using a model with treatment as a fixed effect and bioassay and treatment × bioassay as random effects. For the spring 2018 field experiment, whole-plant aphid numbers averaged on a per plot basis were compared using a model with treatment as a fixed effect and block as a random effect. For the fall 2018 and spring 2019 experiments, aphid numbers per leaf averaged on a per plot basis were compared using a model with treatment, observation date, and the treatment × observation date interaction as fixed effects. Block and treatment × block were random effects to account for the effect of repeated measures (variance component covariance structure). The Kenward-Roger adjustment for denominator degrees of freedom was used to correct for inexact *F* distributions in all models. The Tukey-Kramer adjustment ($\alpha = 0.05$) was used to assist in interpreting pairwise differences in means. When a two-way interaction was significant at $\alpha = 0.05$, the SPLICE and SPLICEDIFF options were used to assist in comparing treatment means at each observation time or date (PROC GLIMMIX, SAS Institute Inc., 2016).

3. Results

3.1. Laboratory experiment

Differences in *M. sacchari* mortality were detected ($F = 33.5$; df = 9, 80.8; $P < 0.001$) among treatments across observation times (Fig. 1). The non-treated control had the lowest mortality [12.3 ± 4.6 (SE) %] whereas the highest mortality was observed on flupyradifurone-treated leaf discs [98.7 ± 2.3 (SE) %]. Mortalities for the pre-mix of *B. bassiana* +pyrethrins [94.2 ± 4.9 (SE) %], pyrethrins [88.3 ± 6.1 (SE) %], and *C. subtsugae* [79.9 ± 6.7 (SE) %)] were the highest among biological insecticides and were not different from the mortality observed on flupyradifurone-treated leaf discs. *Beauveria bassiana*, azadirachtin, and vetiver oil were associated with intermediate mortality, with 66.3 ± 11.9 (SE) %, 44.8 ± 12.0 (SE) %, and 41.8 ± 8.4 (SE) %, respectively. Mortalities on leaf discs treated with *Burkholderia* spp. and *I. fumosorosea* were not different ($P > 0.05$) than mortality on non-treated discs.

Mortality across treatments generally increased between 6 and 72 h after bioassay initiation ($F = 92.8$; df = 3, 249.9; $P < 0.001$), with mortality in non-treated Petri dishes increasing from 0.0 ± 0.0 (SE) % at 6 h to 20.0 ± 6.5 (SE) % at 72 h (Fig. 1). However, a treatment by observation time interaction was detected ($F = 9.3$; df = 27, 250.6; $P < 0.001$), indicating that the effects of treatments varied with observation time (Fig. 1). Flupyradifurone, pyrethrins, the pre-mix of *B. bassiana* + pyrethrins, and *C. subtsugae* showed substantial effects on the aphids starting 6 h after bioassay initiation and mortality increased by 5.4–16.8% 72 h after bioassay initiation. However, the remaining treatments were associated with relatively low mortality at 6 h. Mortality subsequently increased by a minimum of 72.0% for *B. bassiana* and a maximum of 34.2-fold for azadirachtin at 72 h (Fig. 1).

3.2. Greenhouse experiments

The number of *M. sacchari* infesting sorghum plants differed ($P < 0.05$) among treatments in the two greenhouse experiments (Table 2). In the first experiment (aphid-infested plants sprayed), aphid infestation levels decreased by 100% on flupyradifurone-treated plants relative to

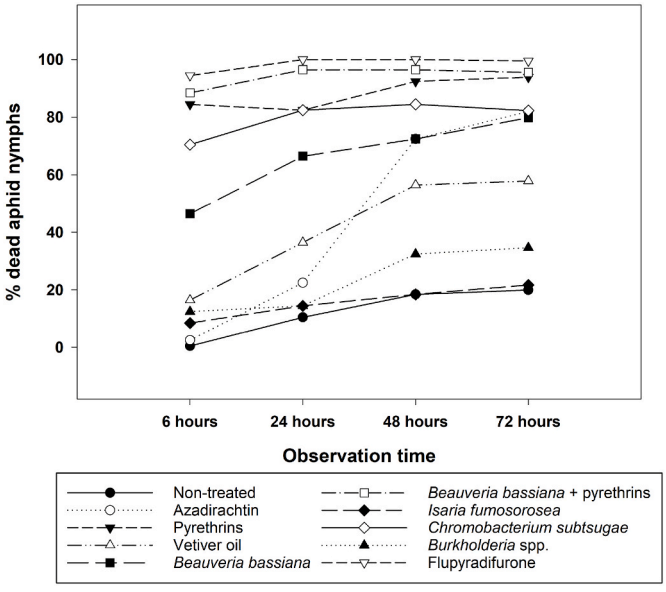

Fig. 1. *Melanaphis sacchari* mortality (means) over time as affected by the application of seven biological insecticides, vetiver oil, and a conventional insecticide under laboratory conditions, summer 2018, Belle Glade, FL.

Table 2
Melanaphis sacchari infestation levels on sorghum plants as affected by the application of commercial biological insecticides and vetiver oil in the greenhouse, summer 2018, Belle Glade, FL. In the first experiment, treatments were applied to sorghum plants infested with aphids. In the second experiment, treatments were applied to sorghum plants before infestation with aphids.

Treatment	First experiment	Second experiment
	No. aphids/plant[a] (Means ± SE)	No. aphids/plant[a] (Means ± SE)
Non-treated	59.3 ± 10.8a	61.7 ± 13.3a
Azadirachtin	1.1 ± 1.5c	1.9 ± 1.7c
Pyrethrins	3.9 ± 3.9c	21.6 ± 9.5bc
Vetiver oil	48.1 ± 13.6ab	58.7 ± 13.1a
Beauveria bassiana	24.9 ± 13.2bc	43.7 ± 12.0ab
Beauveria bassiana + pyrethrins	10.5 ± 6.2c	47.7 ± 13.0ab
Isaria fumosorosea	45.3 ± 9.7ab	34.5 ± 12.7abc
Chromobacterium subtsugae	46.8 ± 15.7ab	54.1 ± 16.6ab
Burkholderia spp.	51.6 ± 12.1ab	51.9 ± 14.9ab
Flupyradifurone	0.0 ± 0.0c	0.0 ± 0.0c
F	12.9	8.6
df	9, 36	9, 36
P > *F*	<0.001	<0.001

[a] Means with the same letter in a column are not significantly different (Tukey-Kramer adjustment, $\alpha = 0.05$).

non-treated plants. Plants treated with azadirachtin, pyrethrins, and the *B. bassiana* + pyrethrins pre-mix sustained 98.2, 93.4, and 82.3% less aphids, respectively, than non-treated plants (Table 2). In the second experiment (non-infested plants sprayed), flupyradifurone also decreased aphid infestation levels by 100%. Azadirachtin and pyrethrins were the only other treatments with measurable effects on *M. sacchari* infestations, with 96.9 and 65.0% less aphids, respectively, than the non-treated plants (Table 2). Although the pre-mix of *B. bassiana* + pyrethrins was associated with lower aphid infestation levels than the non-treated control in the first experiment, differences were not detected ($P > 0.05$) in the second experiment (Table 2).

3.3. Field experiments

Pre-treatment infestation levels averaged 1.0 *M. sacchari*/plant for the spring 2018 experiment, and 15.2 and 2.2 *M. sacchari*/leaf for the fall 2018 and spring 2019 experiments, respectively. Differences in *M. sacchari* infestation levels were detected ($P < 0.05$) among treatments for the single post-treatment observation in spring 2018 and across the three post-treatment observations in spring 2019 (Table 3). In spring 2018, *M. sacchari* infestation levels in flupyradifurone-treated plots were 95.0% lower than in azadirachtin-treated plots. In spring 2019, infestation levels in flupyradifurone-treated plots were 99.1% lower than in *I. fumosorosea*-treated plots. However, aphid infestation levels in non-sprayed plots were not different than those in any treated plots in the two experiments (Table 3). In the fall 2018 experiment, although differences were not detected ($P > 0.05$) among treatments, flupyradifurone-treated plots sustained the lowest *M. sacchari* infestation levels, consistent with the spring 2018 and 2019 experiments (Table 3).

Melanaphis sacchari infestation levels across treatments increased ($P < 0.05$) between the second and third weekly post-treatment observation dates for the fall 2018 and spring 2019 experiments (Table 3). In addition, a treatment by observation date interaction was detected ($F = 2.1$; df = 16, 54; $P = 0.024$) for the spring 2019 experiment. Although infestation levels were not different among treatments for the first and second post-treatment observation dates, differences were detected ($P < 0.05$) for the third date. However, *M. sacchari* infestation levels in non-sprayed plots were not different than those in any other treated plots for that date (Fig. 2).

Table 3
Melanaphis sacchari infestation levels as affected by treatment and post-treatment observation date in three field experiments, 2018–2019, Belle Glade, FL.

Treatment	Spring 2018	Fall 2018	Spring 2019
	No. aphids/plant[a] (Means ± SE)	No. aphids/leaf[a] (Means ± SE)	No. aphids/leaf[a] (Means ± SE)
Non-sprayed	14.6 ± 3.4ab	38.0 ± 13.5a	10.4 ± 5.4ab
Azadirachtin	20.2 ± 4.9a	14.8 ± 9.4a	7.7 ± 5.8ab
Pyrethrins	–	6.8 ± 4.0a	14.0 ± 9.2ab
Beauveria bassiana	13.3 ± 4.1ab	47.8 ± 28.8a	14.2 ± 8.5ab
Beauveria bassiana + pyrethrins	12.3 ± 5.5ab	47.3 ± 27.5a	15.1 ± 9.8ab
Isaria fumosorosea	10.0 ± 1.5ab	–	25.3 ± 19.5a
Chromobacterium subtsugae	16.1 ± 2.6ab	46.5 ± 31.5a	12.6 ± 6.5ab
Burkholderia spp.	14.4 ± 2.2ab	35.0 ± 21.1a	8.9 ± 4.5ab
Flupyradifurone	1.0 ± 0.1b	0.5 ± 0.2a	0.2 ± 0.2b
F	2.8	1.7	3.1
df	7, 21	7, 21	8, 24
P > *F*	0.031	0.163	0.016
Observation date			
First	–	16.6 ± 2.4b	2.9 ± 0.4b
Second	–	20.5 ± 4.8b	5.9 ± 1.1b
Third	–	51.6 ± 11.2a	27.4 ± 4.3a
F	–	15.9	37.5
df	–	2, 48	2, 54
P > *F*	–	<0.001	<0.001

[a] Means with the same letter in a column are not significantly different (Tukey-Kramer adjustment, $\alpha = 0.05$).

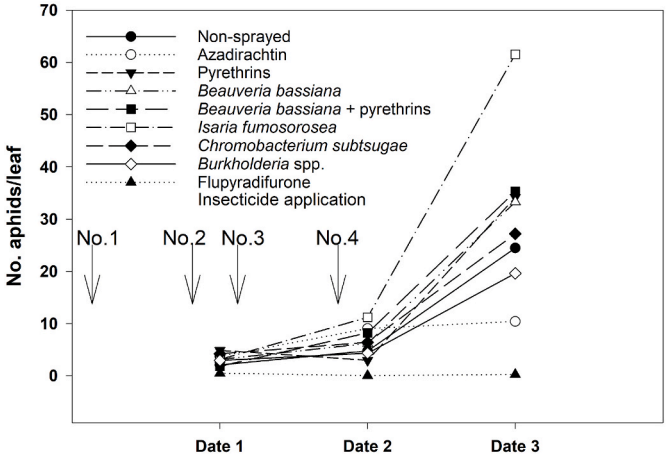

Fig. 2. *Melanaphis sacchari* infestation levels (means) in a sorghum field experiment evaluating seven biological insecticides at three weekly post-treatment observation dates, spring 2019, Belle Glade, FL. Arrows represent insecticide applications.

4. Discussion

Our study, to the best of our knowledge, is the first to provide a comprehensive assessment of seven commercial biological insecticides and one plant essential oil with insecticidal activity that may benefit sorghum farmers in the United States and Haiti. Since the emergence of *M. sacchari* as a major sorghum pest in North America, farmers have relied on a limited number of conventional insecticides with similar modes of action to manage the aphid (Bowling et al., 2016; IRAC (Insecticide Resistance Action Committee), 2020). However, biological insecticides may represent an additional management tactic to combat this pest because they have multiple modes of action (Marrone, 2019) and can be effective aphicides (Stark and Walter, 1995; Kraiss and

Cullen, 2008; Maketon et al., 2013; Kuhar and Doughty, 2016).

Azadirachtin and pyrethrins negatively affected *M. sacchari* infestations in the laboratory and greenhouse experiments. These results are consistent with insecticidal activity observed on numerous insect pests in previous laboratory and field studies (Kraiss and Cullen, 2008; Niklova, 2016; Morehead and Kuhar, 2017). More specifically, a commercial formulation of azadirachtin decreased *M. sacchari* infestations in grain sorghum below damaging levels in a 2-year field study (Díaz-Nájera et al., 2018) although the botanical insecticide was ineffective in another field study (Buntin and Roberts, 2016). In the laboratory experiment, pyrethrins caused >80% mortality at 6 h after assay initiation whereas azadirachtin caused comparable mortality at 72 h after assay initiation. These observations suggest that azadirachtin has delayed effects on *M. sacchari* relative to pyrethrins, likely because of repellent and growth regulator activity (Isman, 2006; Kraiss and Cullen, 2008). In the greenhouse experiments, mainly adult aphids were observed on azadirachtin-treated plants sustaining low aphid infestation levels (W. Calvin, personal observations), suggesting that azadirachtin might have an effect on the development and reproductive capability of *M. sacchari*. Previous studies showed that neem seed oil and azadirachtin cause nymphal mortality, prolong developmental time, and reduce fecundity of *A. glycines* (Kraiss and Cullen, 2008). In contrast, pyrethrins have immediate effects on insects. However, these effects were more pronounced in the greenhouse experiments when pyrethrins were directly applied to *M. sacchari* infesting sorghum plants than when pyrethrins were applied to plant surfaces before aphid infestation. These results suggest that direct exposure of *M. sacchari* to pyrethrins is needed to maximize efficacy in the field. Although results of field experiments were not conclusive in supporting insecticidal activity observed in the laboratory and the greenhouse, azadirachtin and pyrethrins should be included in future research efforts evaluating the role of biological insecticides in *M. sacchari* management.

Vetiver oil caused nearly 60% *M. sacchari* mortality at 72 h in the laboratory experiment but *M. sacchari* infestation levels on vetiver oil-treated sorghum plants were comparable to those on the non-treated control in the greenhouse experiments. The mode of action of vetiver oil against *M. sacchari* is unknown. However, vetiver oil repellency and toxicity to several arthropod pests have been observed (Zhu et al., 2003; Henderson et al., 2005a, 2005b). Haiti is the first vetiver oil producer worldwide (Belhassen et al., 2015) and large quantities of byproducts are produced daily. These byproducts may contain some levels of vetiver oil and may serve as a potential low-cost insecticide to smallholders in Haiti. In addition, intercropping vetiver grass with other crops may cause deleterious effects to pest infestations. For instance, the spotted stem borer, *Chilo partellus* (Swinhoe), prefers to oviposit on vetiver grass, which assists in controlling the insect because of a decrease in offspring survival (Van den Berg et al., 2003). Thus, the use of vetiver oil, byproducts of vetiver oil production, or vetiver grass should be further studied and might play a role in *M. sacchari* management in agroecosystem where vetiver is widely available such as in Haiti.

Beauveria bassiana strain GHA negatively affected *M. sacchari* in the laboratory and greenhouse experiments. However, this effect occurred to a lesser extent than in a previous laboratory study showing that *B. bassiana* CKB-48 caused nymphal mortality approaching 100% (Maketon et al., 2013). The pre-mix of *B. bassiana* + pyrethrins was among the most effective biological insecticides in the laboratory and greenhouse experiments. This pre-mix negatively affected *M. sacchari* to a greater extent than did *B. bassiana* alone in spite of the reduced rate of *B. bassiana* and pyrethrins in the pre-mix. Similarly, Reddy and Antwi (2016) showed that *B. bassiana* applied alone was less effective against wheat head armyworm, *Dargida diffusa* (Walker), larvae than when mixed with plant extracts, indicating that *B. bassiana* is more effective when combined with other insecticides. The other fungus, *I. fumosorosea*, did not have measurable effects on *M. sacchari* in any experiment of our study. In field studies, *I. fumosorosea* did not decrease *M. sacchari* infestations in grain sorghum (Harris-Shultz et al., 2020). Thus, although *I. fumosorosea* has negative effects on selected insect pests (Hunter et al., 2011), the further study of this biological insecticide for *M. sacchari* management in sorghum should not be prioritized. In contrast, further research on *B. bassiana* alone or in combination with other biological insecticides such as pyrethrins is warranted in spite of inconclusive results under field conditions.

The bacterial insecticide *C. subtsugae* showed adverse effects on *M. sacchari* only in the laboratory experiment whereas *Burkholderia* spp. did not show measurable effects in any of the experiments of our study. It is likely that the activity of *C. subtsugae* is limited to narrow environmental conditions because in a previous field experiment, *C. subtsugae* was also ineffective against *M. sacchari* (Studebaker and Jackson, 2017). Nonetheless, other studies showed that *C. subtsugae* can be deleterious to numerous pest species including aphids (Shapiro-Ilan et al., 2013; Andon and Shetlar, 2015; Kuhar and Doughty, 2016). To the best of our knowledge, *Burkholderia* spp. has not been previously tested on any aphid species although previous studies showed that *Burkholderia* spp. can have adverse effects on *A. vaccinia* and *S. exigua* (Cordova-Kreylos et al., 2013; Wise et al., 2015). Thus, our results suggest that the further study of *C. subtsugae* and *Burkholderia* spp. for *M. sacchari* management in sorghum should not be prioritized.

The conventional insecticide, flupyradifurone, was used as a standard treatment in our study because of documented efficacy against *M. sacchari* in field evaluations conducted in the United States (VanWeelden et al., 2016; Buntin et al., 2018; Zarrabi et al., 2018; Owens et al., 2020). In our study, flupyradifurone was effective across the laboratory, greenhouse, and field experiments. This insecticide was consistently associated with the highest aphid mortality or lowest aphid infestation levels although differences with other treatments were not always detected. In contrast, the efficacy of biological insecticides was not consistent across the experiments. Some biological insecticides showed high efficacy in the laboratory experiment with intermediate efficacy in the greenhouse experiments and little to no efficacy in the field experiments, which is consistent with Dorschner et al. (1991) or Edelson et al. (2002). This decrease in biological insecticide efficacy might be partially associated with a difference in spray coverage because the sorghum leaves in the field might receive less spray solution than the sorghum leaf discs used in the laboratory experiment and the potted sorghum plants used in the greenhouse experiments. In addition, the environmental conditions to which the insecticides were exposed in the greenhouse and the field likely decreased their efficacy. Solar radiation, microbial activity, rainfall, and temperature are factors that influence the degradation and efficacy of biological insecticides (Copping and Menn, 2000). Pyrethrin-I, which is a main component of pyrethrins, breaks down rapidly on plant surfaces when exposed to sunlight with a half-life under field conditions of 8–14 h on potato (*Solanum tuberosum* L.) leaves, <3 h on pepper (*Capsicum annuum* L.) leaves, and <1 h on tomato (*Solanum lycopersicum* L.) leaves (Antonious et al., 2001; Antonious, 2004). The half-life of pyrethrin-I applied to peaches [*Prunus persica* (L.) Batsch] is 2.5 days under field conditions (Angioni et al., 2005). Azadirachtin is also subject to rapid degradation by sunlight and microbial activity with a half-life under field conditions of <1 day on olives (*Olea europaea* L.) and 2.5 days on castorbean (*Ricinus communis* L.) leaves (Caboni et al., 2002; Johnson et al., 2003). However, azadirachtin can be active up to 21 days after treatment compared to 35 days after treatment for cyfluthrin, tebufenozide, and diflubenzuron (Webb et al., 1998). Similar to pyrethrins and azadirachtin, *B. bassiana* is susceptible to ultra-violet radiation (Inglis et al., 1995). In addition, rainfall decreases the number of *B. bassiana* conidia on plant surfaces although emulsifiable suspensions such as the BotaniGard® ES used in our study are considered relatively rainfast (Inglis et al., 2000). A previous laboratory study also showed that *B. bassiana* has greater insecticidal activity on the differential grasshopper, *Melanoplus differentialis* (Thomas), at temperatures between 15 °C and 25 °C whereas temperatures have little impact on azadirachtin effectiveness (Amarasekare and Edelson, 2004). Thus, while experiments in our study provided a diversity of sunlight,

temperature, relative humidity, and rainfall conditions, these environmental conditions in the greenhouse and field experiments might not have been optimal for the performance of the biological insecticides. In addition, reinfestation of plots by *M. sacchari* and predation from natural enemies might have further prevented the detection of effects associated with the biological insecticides in the field experiments.

Results of our study suggest that biological insecticides including pyrethrins, azadirachtin, *B. bassiana*, and the pre-mix of *B. bassiana* +pyrethrins might control *M. sacchari* infestations in sorghum if applied under favorable environmental conditions with good coverage. Therefore, further studies should address application methods, environmental conditions, and rates conducive to optimal efficacy of these insecticides in the field. The use of these biological insecticides in a pest management program for *M. sacchari* would allow reduced-risk sorghum production and insecticide resistance mitigation. The use of these insecticides would also support organic sorghum production.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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