Parasitization of the sugarcane aphid, Melanaphis sacchari, *by commercially available aphid parasitoids*

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Parasitization of the sugarcane aphid, *Melanaphis sacchari*, by commercially available aphid parasitoids

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Abstract Identification of natural enemies of novel pests is important for the development of effective integrated pest management. Commercially available parasitoids used for control of arthropod pests have potential for enhancing biological control of invasive pests. The sugarcane aphid, *Melanaphis sacchari* (Hemiptera: Aphididae), is a new pest on sweet sorghum, *Sorghum bicolor*, in the USA. Surveys of *M. sacchari* have not detected any parasitoids in central Kentucky. In North America, *Aphelinus abdominalis* (Hymenoptera: Aphelinidae), *Aphidius ervi* (Hymenoptera: Braconidae), *Aphidius colemani,* and *Aphidius matricariae* are sold for aphid management. This study's objective was to determine the host acceptance and suitability of *M. sacchari* for these parasitoid species. Host acceptance was assessed by counting attacks and oviposition strikes by parasitoids on *M. sacchari*. All parasitoid species accepted *M. sacchari* as a host in the parental generation (purchased adults) and the F1 generation (reared from *M. sacchari*). Host suitability was evaluated by transferring *M. sacchari* from host acceptance trials to caged sweet sorghum plants. Cages were monitored for aphid mummies and emerged adult parasitoids. Parental *A. colemani* produced the most mummies and adult parasitoids and reduced final *M. sacchari* numbers by 75%. *A. ervi* had a similar impact on *M. sacchari* populations but produced fewer mummies and adults. *A. matricariae* and *A. abdominalis* did not reduce *M. sacchari* populations. F1 parasitoids produced few adults and did not reduce *M. sacchari* populations. *A. colemani* demonstrated potential for field releases with the ability to use *M. sacchari* as a host and reduce aphid population growth.

Keywords Parasitoid wasp · Host acceptance · Host suitability · Hemiptera · Aphididae · Hymenoptera

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Introduction

As new insect pests emerge, identifying natural enemies for biological control is critical. Classical biological control could provide the ideal parasitoid-host match for a new invasive pest, however, this requires surveys in the pest's native region, non-target assessment and legal approval (van Lenteren et al. 2006; de Clercq et al. 2011). Fortunately, mass-reared aphid parasitoids are available, and can be used in

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augmentative releases to manage aphid pests, e.g., *Aphidius colemani* Viereck (Hymenoptera: Braconidae) for *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Vasquez et al. 2006) or *Aphidius ervi* Haliday for *Aulacorthum solani* Kaltenbach (Hemiptera: Aphididae) (La-Spina et al. 2019). In North America, four species of aphid parasitoids are used for augmentative releases: *Aphelinus abdominalis* Dalman (Hymenoptera: Aphelinidae), *A. colemani, A. ervi*, and *Aphidius matricariae* Haliday. The four species have relatively broad host ranges, spanning 18, 12, ten and nine aphid genera for *A. abdominalis, A. colemani, A. ervi* and *A. matricariae,* respectively (Stary et al. 1993; Japoshvili and Abrantes 2006; Tomanović et al. 2009). Prior to recommending augmentative releases of these parasitoids, testing to demonstrate their effectiveness at managing the target pest is required.

The first step to determine the viability of a parasitoid species for augmentative biological control of a new aphid pest species is to determine host acceptance and host suitability for the parasitoid. Host acceptance is defined as the parasitoid choosing to oviposit in the aphid (Vinson 1976), and host suitability is defined as the successful development of parasitoids in the aphid host (Vinson and Iwantsch 1980). Host acceptance can be influenced by several factors, including host plant, visual stimuli, olfactory cues and cues detected by the ovipositor upon insertion into the potential host (Vinson 1976; Messing and Rabasse 1995; Larocca et al. 2007). Once an egg has been laid within a host, parasitoid development (host suitability) can depend on the host's immune response, endosymbionts, nutritional state and age (Vinson and Iwantsch 1980; Cayetano and Vorburger 2015). High host acceptance and suitability can enable parasitoids to persist for multiple generations and increase their value as biological control agents. The host species used to rear parasitoids in commercial insectaries is unlikely to be the same species as the pest they are purchased to control. Second generation parasitoids reared on the target pest species may differ from their parents in their fitness, host acceptance and suitability (Pennacchio et al. 1994; Henry et al. 2008).

The sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), is a cosmopolitan pest that feeds on several genera of grasses (Singh et al. 2004). *M. sacchari* has been present in the USA as an occasional pest of sugarcane since the 1970s (Denmark 1988). In 2013, a new genotype of *M. sacchari* was found infesting grain, forage, and sweet sorghum in the southern USA (Nibouche et al. 2018), resulting in major crop losses (Bowling et al. 2016; Brewer et al. 2017). *M. sacchari* reached Kentucky in 2015, where it infested sweet sorghum fields and caused crop failures and yield losses (Villanueva 2016). Sweet sorghum is predominately grown for syrup and is an important crop in Kentucky, worth an estimated $16–27 million yearly (R. Bessin, personal observation). Once established on sorghum, *M. sacchari* populations can increase rapidly and reduce sugar concentrations. A single aphid can produce 98 nymphs during their 2–5 week adult life span (Hinson 2017).

Management recommendations for *M. sacchari* in Kentucky sweet sorghum are limited. Chemical recommendations include insecticidal soap or flupyradifurone (Sivanto® Prime, Bayer CropScience, Leverkusen, Germany), which is currently only available under an emergency exemption. Cultural management practices include early planting and harvesting prior to the development of large *M. sacchari* infestations in the latter half of the sweet sorghum growing season. *M. sacchari* is attacked by a variety of natural enemies (Maxson et al. 2019). *Aphelinus nigritus* Foerster (Hymenoptera: Aphelinidae) and *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae) are two parasitoid species which attack *M. sacchari* in the USA (Maxson et al. 2019), neither are commercially avaliable. Although both *L. testaceipes* and the *Aphelinus varipes* species complex (which includes *A. nigritus*) (Wharton 1983) occur in Kentucky, USA (Lenhart and White 2017), *M. sacchari* mummies have only been observed in western Kentucky (R. Villanueva, personal communication). *M. sacchari* is believed to originate from Asia (Nibouche et al. 2018) where it is attacked by several parasitoids (Singh et al. 2004).

Existing host records for the four commercially available aphid parasitoids show promise for their ability to parasitize *M. sacchari.* Many naturally occurring parasitoids including *A. colemani* and *A. ervi* parasitize *M. sacchari* in the central Mexican state of Guanajuato (Salas-Araiza et al. 2017), but a survey in Coahuila, Mexico (which borders the USA) found only *L. testaceipes* and *A. nigritus* (García-González et al. 2018). *A. abdominalis* and *A. matricariae* are not known to attack aphids in the genus *Melanaphis*, but

do have hosts within the same sub-tribe (Rhopalosiphina) (Kavallieratos et al. 2004; Japoshvili and Abrantes 2006; Kim and Lee 2008). Parasitoids are an important part of aphid population suppression (Snyder and Ives 2001, 2003; Schmidt et al. 2003) and their addition to the sweet sorghum system may reduce *M. sacchari* population growth and abundance. Our objectives were to determine the host acceptance and suitability of *M. sacchari* for *A. abdominalis, A. ervi, A. colemani,* and *A. matricariae* and to assess effects of these parasitoids on the population growth of *M. sacchari*. We tested two generations of parasitoids and compared fitness of insectary reared wasps to their offspring reared on *M. sacchari*. The offspring's fitness can predict if inoculative or inundative releases will be needed for pest management.

Materials and methods

Insect and plant rearing

M. sacchari colonies were established from field collections at Spindletop Research Farm (University of Kentucky, Lexington, KY, USA) in 2018 and maintained at 25 °C, 14:10 L:D in growth chambers (Percival Scientific Inc, Perry, IA, USA) on four to eight-week-old sweet sorghum (*Sorghum bicolor*, variety Dale). Sweet sorghum was grown in 20 cm pots with ~ 15 plants per pot at 24 °C in greenhouses (natural light supplemented with mercury halide lamps set at 14:10 L:D) and fertilized every two weeks with 20–20–20 fertilizer (Southern States, Richmond VA) at a rate of 1.6 g l^{-1}.

A. abdominalis, *A. ervi*, *A. colemani,* and *A. matricariae* were obtained from a commercial insectary (Rincon-Vitova Insectaries, Inc. Ventura, CA, USA). Upon delivery, parasitoid pupae were kept at 22 °C, 14:10 L:D in growth chambers. Cotton balls soaked in a 1:1 water:honey solution were supplied for adult parasitoids.

Experimental design

Host acceptance and suitability of *M. sacchari* was assessed with four parasitoid species (treatments): *A. abdominalis*, *A. ervi*, *A. colemani* and *A. matricariae*. Host acceptance was determined by behavioral observations of parasitoid-*M. sacchari* interactions over 24 h. Host suitability of *M. sacchari* for each treatment was assessed with aphids from host acceptance trials. These aphids were monitored for development of mummies and emergence of adult parasitoids, and results were compared to a control group of *M. sacchari* that was not exposed to wasps. In addition, *M. sacchari* populations were counted to assess the biological control potential of each parasitoid species relative to each other and to a control set of *M. sacchari* that was not exposed to parasitoids.

Host acceptance

Host acceptance trials were initiated 72 h after the first adult parasitoids emerged from the shipped pupae. This 72 h period allowed for emergence and mating: all females were assumed to have mated within this time. For this experiment, the parasitoid generation received from the insectary was considered the parental generation. Arenas for observing host acceptance behaviors were plastic Petri dishes (10 × 1.5 cm) lined with a moist paper towel. A 1 cm hole covered with mesh (530 micron mesh) in the Petri dish lid provided ventilation. Three 8 × 1 cm sections of sweet sorghum leaf with ten M. *sacchari* per section were placed in each dish. Infested leaves contained a variety of *M. sacchari* life stages as the parasitoid life stage preference for *M. sacchari* is unknown. A single female parasitoid was introduced to each dish. Host acceptance was replicated 12 times per parasitoid species. The control treatment for host suitability was also initiated at this time, replicated 12 times, and treated the same as other treatments but without receiving a female parasitoid. Five-minute observations were made immediately following addition of a female parasitoid, and at 8 and 24 h after parasitoid introduction. The number of attacks (striking at *M. sacchari* with their ovipositor, but not inserting their ovipositor into an aphid) and ovipositions (inserting their ovipositor into an aphid) for each parasitoid female was recorded. Ovipositions were assumed to successfully deposit an egg in *M. sacchari*: no dissections were performed to determine presence of parasitoid eggs. Arenas were placed in a growth chamber (25 °C, 14:10 L:D) after the first and second observation. Following the third observation, host suitability trials were initiated.

Host suitability

Host suitability was examined in 60 × 60 × 60 cm cages (bugDorm, Megaview Science Inc., Talchung, Taiwan) containing a 10 cm pot with three eight week old sweet sorghum plants. Sweet sorghum was grown as before. Cages were kept in greenhouses (25 °C, 14:10 L:D). Leaf sections infested with *M. sacchari* from host acceptance arenas were placed on sweet sorghum in the host suitability testing cages, one arena per cage. Due to space limitations, 11 of the 12 host acceptance replicates per treatment were randomly selected for use in the host suitability trial. This yielded 11 replicates per treatment, and 55 total cages. Parasitoid females used in the host acceptance experiment were removed. The number of *M. sacchari* in cages were counted every other day for ten days and mummies and adult parasitoids were counted every other day for 16 days. Emerged adult parasitoids (F1) were removed from cages with an aspirator and stored in species specific growth chambers at 25 °C and 14:10 L:D. F1 adult parasitoids were provided cotton balls soaked in a 1:1 water:honey solution.

F1 generation

On day 13 of the parental host suitability trials, five F1 female parasitoids from each species were collected and tested for host acceptance and suitability in the same manner as the parental parasitoid generation. There were five replicates per species (parental *A. abdominalis* did not produce any progeny and were not included in F1 trials). Sweet sorghum plants used in the host suitability trials were ten weeks old. Representative adults from parental, F1 and F2 were collected for measurement of head capsule width to compare variation in size using a stereoscope at 50 × magnification. These representatives did not include the adult females used in host acceptance trials. Head capsule size is correlated with egg size and load in two other Braconidae species, *Aphaereta minuta* Nees (Visser 1994) and *Lysiphlebus fabarum* Marshall (Ameri et al. 2013). Measurement of head capsule width was done in ImageJ (Schindelin et al. 2012). Sex of parasitoids could not be determined for head capsule measurements as they were stored in a manner that did not preserve the abdomen sufficiently to identify the presence of the ovipositor.

Data analysis

Fisher's exact test was used to compare the ratio of female parasitoids that had at least one attack or one oviposition to those that did not among all treatments. The number of attacks and ovipositions made by females was not analyzed due to uneven and low occurrences among the species. Differences in life stage distribution of *M. sacchari* in the host acceptance trials were assessed with a contingency table for both parental and F1 generations. *M. sacchari* mummies were most abundant eight days after parasitoid exposure for all parasitoid species in both generations. After day eight, mummy counts began to decline slightly, likely due to mummies falling off leaves. Day eight was therefore chosen for analysis of mummies.

Number of mummies observed eight days after exposure to parasitoids and total adult parasitoids emerged were analyzed as a proportion of the original number of *M. sacchari* exposed to parasitoids during the host acceptance trials. This was done to account for the slight variation in *M. sacchari* used in the host acceptance trials, more or less two aphids per arena. Both mummies and adults proportions were analyzed with a generalized linear model (GLM) using a binomial distribution and a logit link function. Species was a fixed effect and Tukey's adjustment was used to compare differences among treatments. Controls and *A. abdominalis* treatments were not included in mummy or adult parasitoid emergence analysis because they were all zeros (with exception of one mummy from one *A. abdominalis*). Difference in head capsule size among generations was assessed separately for each species. Head capsule size was compared with an ANOVA separately for each species, with generation as a fixed effect. Sex was not determined: all individuals were therefore pooled within a generation.

Change in *M. sacchari* population size over time was assessed in a repeated measures generalized linear model with a negative binomial distribution and log link. Day and treatment were fixed effects and cage was a random effect. *M. sacchari* abundance on day 10 (final day of *M. sacchari* counts) was also assessed in a generalized linear model with a negative binomial distribution and a log link function. Treatment was a fixed effect. Differences between treatments was determined with Tukey's test. Host acceptance data was assessed in R v. 3.2.4 (R Core Team 2016).

Generalized linear models (PROC GLIMMIX) and contingency tables (PROC FREQ) were performed in SAS (SAS Institute Inc 2014). Voucher specimens of all four parasitoid species are in the University of Kentucky Insect Museum. Data are deposited in the UKnowledge repository https://doi.org/10.13023/a4fv-f708.

Results

Host acceptance

All parasitoid species in the parental generation had one or more females attack and oviposit in *M. sacchari* during the 15 min of observations (Table 1). Number of individuals that attacked at least once differed among species (Fisher's exact test, $p = 0.06$). *A. colemani* had the highest number and *A. abdominalis* had the lowest number of individuals attacking at least once. The life stage distribution of the 30 *M. sacchari* used in the parental host acceptance differed by among treatment ($\chi^2 = 43.14$, df = 20, $p = 0.002$). This difference was due to control arenas having fewer alate ($\chi^2 = 11.78$, df = 4, $p = 0.02$) and 4th instar ($\chi^2 = 10.16$, df = 4, $p = 0.04$) *M. sacchari* than the other treatments. *A. abdominalis* did not produce any adults from the parental generation and was therefore not tested during the F1 generation. The life stage distribution of the 30 *M. sacchari* used in the F1 host acceptance did not differ among treatments ($\chi^2 = 22.34$, df = 15, $p = 0.10$). The F1 generation of the other four species of parasitoids attacked and oviposited in *M. sacchari* at least once (Table 1). The proportion of F1 individuals that attacked (Fisher's exact test, $p = 0.07$) and oviposited (Fisher's exact test, $p = 0.07$) at least once differed among species, with *A. colemani* having the highest proportion. The number of attacks and ovipositions was not statistically analyzed among the F1 generation due to the uneven number of individuals that attacked among parasitoid species. However, *A. colemani* had the highest number of attacks and ovipositions over the 15 min of observation in the F1 generation.

Host suitability

All parental and F1 generations of *Aphidius* species produced mummies and adults (Table 2). Mummies were first found six days after aphid exposure to parasitoids in both generations for the *Aphidius* species. Treatments in the parental generation differed in the number of mummies present eight days after exposure to parasitoids (GLM $F_{2,30} = 11.11$,

Table 1 Host acceptance of *M. sacchari* by *A. colemani, A. ervi, A. matricariae* and *A. abdominalis* parental and F1 generations. Parental generation had 12 replicates per species

Generation	Species	Attacked		Oviposited	
		% ±SE	Mean ±SE	% ±SE	Mean ±SE
Parental	*A. colemani*	58±14	7.4±2.5	42±14	4.8±1.9
	A. ervi	33±14	3.0±1.0	17±11	2.0±1.0
	A. matricariae	17±11	12.0±6.0	25±13	8.0±3.5
	A. abdominalis	8±8	1.7±0.7	25±13	1.0±0
	Fisher's exact test	0.06	–	0.67	–
F1	*A. colemani*	100±0	9.4±3.0	100±0	5.2±1.4
	A. ervi	60±22	4.3±0.9	60±22	2.0±1.2
	A. matricariae	20±18	2.0±0	20±18	1.0±0
	A. abdominalis	NA	NA	NA	NA
	Fisher's exact test	0.07	–	0.07	–

The number of attacks and ovipositions made by females was not statistically analyzed due to uneven and low occurrences among the species. F1 had five replicates per species. % is percentage of the 12 females that attacked or oviposited at least once during the total 15 min of observation. Mean = mean number of attacks or ovipositions of those individuals that attacked or oviposited at least once during the 15 min of observation. NA indicates that there were no individuals to test. Fisher's exact test *p* values for percentage of individuals that attacked or oviposited are displayed at the bottom of their respective columns

Table 2 Host suitability of *M. sacchari* for both parental and F1 parasitoid generations of *A. colemani, A. ervi, A. matricariae* and *A. abdominalis*

Generation	Species	Mummies	Adults	Ratio
Parental	*A. colemani*	12.7 ± 1.8 a	11.1 ± 2.2 a	0.87 ± 0.09
	A. ervi	3.4 ± 0.8 b	2.9 ± 0.6 b	0.85 ± 0.16
	A. matricariae	6.3 ± 1.3 b	3.6 ± 1.1 b	0.57 ± 0.18
	A. abdominalis	0.1 ± 0.1	0	0
F1	*A. colemani*	14.0 ± 1.5 a	4.4 ± 2.2 a	0.31 ± 0.12
	A. ervi	13.6 ± 4.5 a	4.4 ± 2.1 a	0.32 ± 0.56
	A. matricariae	4.0 ± 2.5 a	0.9 ± 0.4 a	0.23 ± 0.04
	A. abdominalis	NA	NA	NA

A. abdominalis was not included in statistical analysis due to low counts. Means followed by the same letter within a generation and column are not significantly different (Tukey: $p < 0.05$). Mean (± SE) cumulative mummies found eight days after *M. sacchari* exposure to parasitoids (Mummies) per cage and total adult parasitoids per cage that emerged from *M. sacchari* 16 days after 30 *M. sacchari* were exposure to parasitoids (Adults). Ratio is the adults parasitoids produced per mummy observed eight days after aphid exposure to parasitoids. NA indicates that there were no individuals to test

$p < 0.001$). *A. colemani* produced more mummies than *A. matricariae* (Tukey's test, $p = 0.02$) or *A. ervi* ($p < 0.001$). Parental *A. abdominalis* produced one mummy from all cages by day eight and a total of four over 16 days of host suitability testing. Eight days after exposure to F1 parasitoids there was a slight difference among the number of mummies produced among treatments ($F_{2,12} = 3.37$, $p = 0.07$). *A. matricariae* had produced slightly fewer mummies on day eight relative to *A. colemani* ($p = 0.07$) and *A. ervi* ($p = 0.08$).

In the parental generation, *A. colemani* and *A. ervi* adults began to emerge ten days and *A. matricariae* 12 days after aphids were exposed to parasitoids. *A. colemani* produced the most adults per cage ($F_{2,30} = 10.92$, $p < 0.001$) over the 16 days of parental host suitability trials. *A. colemani* produced more adult parasitoids than *A. ervi* ($p = 0.001$) and *A. matricariae* ($p = 0.003$). All parental *A. colemani* produced F1 adults and ten of 11 parental *A. ervi* and *A. matricariae* produced F1 adults. F2 parasitoids took two days longer to emerge than their parents. F1 parasitoids did not differ in the amount of adult parasitoids produced ($F_{2,12} = 1.56$, $p = 0.24$). F1 *A. colemani* and *A. ervi* had four of five replicates produce adults. Only one F1 *A. matricariae* produced adult offspring.

The F1 generation produced more mummies but fewer adults than the parental generation (Table 2). Over half of mummies from parental females (based on counts on day eight) developed into adults, whereas only an average of one third of F1 mummies developed into adults. There was a noticeable difference in mummy color within cages and between generations. Brown and black colored mummies were found in both generations for *A. colemani, A. ervi* and *A. matricariae* cages. In the parental generation, 13 ± 3% were black mummies. F1 generation produced 54 ± 3% black mummies among *Aphidius* species cages.

Head capsule width differed between the generations for *A. colemani* (ANOVA $F_{2,55} = 6.22$, $p = 0.003$), *A. matricariae* ($F_{2,44} = 32.75$, $p < 0.001$) and *A. ervi* ($F_{2,44} = 19.01$, $p < 0.001$) (Table 3). F1 *A. colemani* head width was significantly smaller than parental ($p = 0.04$) or F2 ($p = 0.003$). *A. matricariae* F1 head widths were smaller than parental ($p < 0.001$), F2 were not significantly different from parental or F1. *A. ervi* parental head capsules were wider than F1 ($p < 0.001$) or F2 ($p < 0.001$).

Melanaphis sacchari population response

Populations of *M. sacchari* increased over the course of the experiment in parental ($F_{4,200} = 254.3$, $p < 0.001$) and F1 ($F_{4,64} = 173.94$, $p < 0.001$) generations. In both parental ($F_{16,200} = 4.37$, $p < 0.001$) (Fig. 1) and F1 ($F_{12,64} = 2.91$, $p = 0.003$) (Fig. 2) experiments there was a significant interaction between date and treatment, indicating that *M.*

Table 3 Mean head width (mm) of aphid parasitoids from parental, second (F1) and third (F2) generations from *M. sacchari*

Species	Parental		F1		F2	
	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE
A. colemani	24	0.38 ± 0.01a	17	0.36 ± 0.01b	17	0.39 ± 0.01a
A. ervi	23	0.46 ± 0.01a	12	0.37 ± 0.01b	17	0.37 ± 0.01b
A. matricariae	32	0.40 ± 0.01a	13	0.33 ± 0.01b	2	0.37 ± 0.01b
A. abdominalis	19	0.44 ± 0.01	0	NA	0	NA

A. abdominalis was not included in statistical analysis due to low counts. Means with the different letters within a species denote significant differences ($p < 0.05$) according to Tukey's Test

n is number of individuals measured (females and males). NA indicates there were no individuals to measure

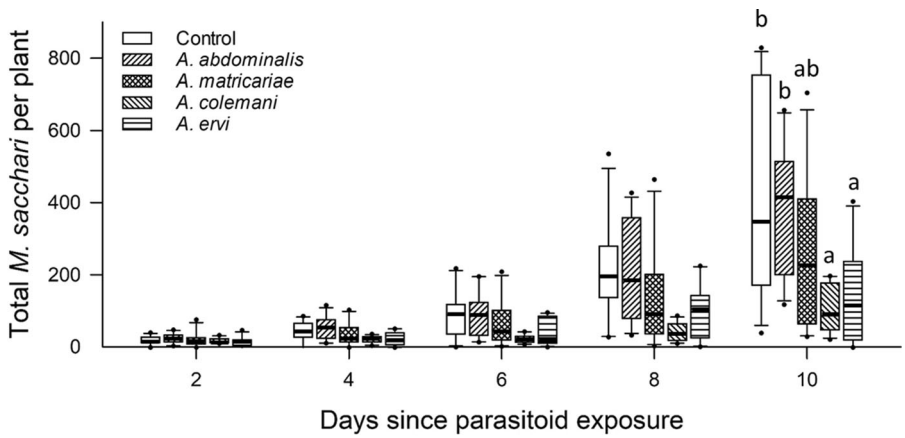

Fig. 1 Boxplot of *M. sacchari* abundance ppopulations differed significantly onost adult parasitoid parental generation exposure on sweet sorghum in greenhouse cages. Thick bars across the boxes are the medians, the upper and lower portions of the boxes are the 75 and 25 percentiles, respectively. Whiskers extend to the 90 and ten percentile of the data. Outliers (solid dots) are data points below the ten percentile or above the 90 percentile. Letters indicate significant difference ($p < 0.05$) according to Tukey's post-hoc test on day ten after *M. sacchari* was exposed to parasitoids

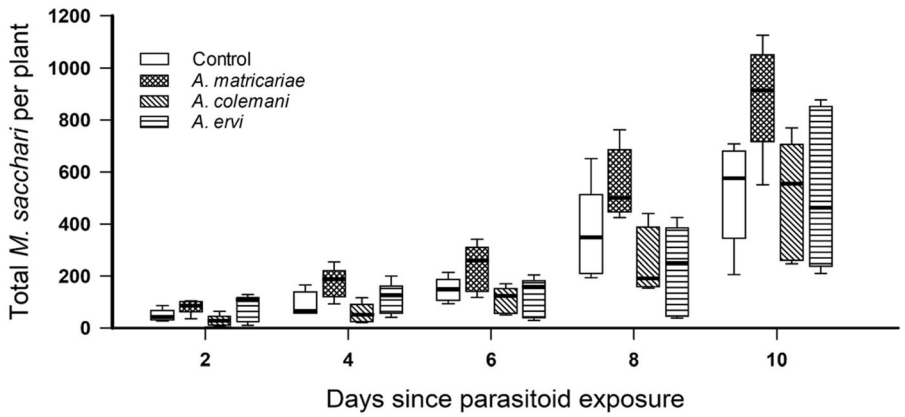

Fig. 2 Boxplot of *M. sacchari* abundance post adult F1 generation exposure on sweet sorghum in greenhouse cages

sacchari population growth patterns differed by treatment. In the parental generation experiment *M. sacchari* populations differed significantly on day ten ($F_{4,50} = 5.68$, $p < 0.001$). *M. sacchari* in control cages grew from 30 to 405 ± 82 aphids per cage after ten days. *M. sacchari* populations grew to 387 ± 54, 263 ± 62, 149 ± 41 and 102 ± 19 after exposure to *A. abdominalis, A. matricariae, A. ervi* and *A. colemani*, respectively. Aphid population sizes when exposed to *A. ervi* ($p = 0.05$) and *A. colemani* ($p = 0.003$) were lower than controls. *M. sacchari* in control cages during the F1 generation experiment increased to 526 ± 88 aphids per cage after ten days. *M. sacchari* populations grew to 887 ± 94, 522 ± 138, 496 ± 102 and when exposed to *A. matricariae, A. ervi* and *A. colemani*, respectively. None of the treatments during the F1 trials differed significantly on day ten from one another ($F_{3,16} = 2.11$, $p = 0.14$).

Discussion

The four species of parasitoids accepted *M. sacchari* as a host, but the host was only suitable for species in the genus *Aphidius*. *A. abdominalis* had the lowest frequency of attacks and ovipositions. Differences in attack rate have previously been observed between *Aphelinus* and *Aphidius*. *A. matricariae* oviposited six times more than *Aphelinus asychis* Walker on *Diuraphis noxia* Mordvilko (Hemiptera: Aphididiae), despite field populations of *A. asychis* being more prevalent in *D. noxia* infested fields (de Farias and Hopper 1999). F1 *A. colemani* and *A. ervi* had an increase in host acceptance from their parents, with more individuals attacking and ovipositing. Increase in host acceptance over generations has been observed in other aphid parasitoids (Pennacchio et al. 1994; Messing and Rabasse 1995; Henry et al. 2008). *A. matricariae* had fewer attacks and ovipostions in the second generation relative to the parental generation, despite having some of the highest number of observed attacks and ovipositions. Ten of the 11 replicates for each parental *Aphidius* species produced mummies, even though not all of the adults were observed to attack *M. sacchari* during host acceptance. Longer observations periods may be necessary to better quantify host acceptance. Parasitoids were given access to a finite number of hosts and multiple eggs may have been laid within one host, resulting in superparasitism. Superparasitism can alter the development of the host, parasitoid larvae and adult parasitoid's host choice (Cloutier and Mackauer 1980; Bai and Mackauer 1992). No aphid dissections were performed to determine if superparasitism occurred. However, host acceptance and suitability data both have similar conclusions, with species that oviposited more producing more adults. Future studies will need to determine whether these parasitoids are laying more than one egg and if multiple eggs effect parasitoid development within *M. sacchari*.

Parental *A. colemani* produced four times more adults than either *A. ervi* or *A. matricariae*, whereas *A. abdominalis* produced no viable adults. It is possible *A. abdominalis* needed longer than the 16 day test period to complete development, as the lifecycle of *A. abdominalis* can range from two to three weeks (Höller and Haardt 1993; Couty et al. 2001; Velasco-Hernández et al. 2017). However, subsequent dissection of mummies produced by *A. abdominalis* found no larvae or pupae within *M. sacchari,* which suggests *M. sacchari* is not a suitable host for *A. abdominalis*. Endosymbionts can provide resistance for their host to natural enemies (Oliver et al. 2003; McLean and Godfray 2015). Screening of *M. sacchari* has found a diverse array of endosymbionts (Holt et al. 2020), however, the exact impact on parasitoid-host interactions these endosymbionts may play has not been explored.

Because chemical control measures for *M. sacchari* are often applied only a few weeks before harvest, a delay in aphid population growth could be the difference between needing to spray or not. Aphid mortality from mummy formation is not the only means of suppressing aphid population growth. *A. ervi* produced a quarter of the mummies and adults compared with *A. colemani*, yet suppressed *M. sacchari* populations equally. Ingerslew and Finke (2017) found that the act of stinging *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) by *Aphidius ervi* shortened the aphid's lifespan, regardless of whether a parasitoid egg was deposited. Unlike their parents, F1 *A. colemani* and *A. ervi* failed to suppress *M. sacchari* populations. This difference is unexpected as F1 produced more mummies than parental, which would normally mean fewer aphids able to reproduce. We are unsure what may be causing this difference between generations. A possibility is that the F1 had a lower

fitness (based on their head size) and did not suppress *M. sacchari*'s immune system, which hindered the F2 larvae and allowed the aphids to produce more offspring. Another possibility is that *M. sacchari* responded to the parasitoid attacks with an increase in reproduction, as has been observed in *Aphis glycines* Matsumura (Kaiser and Heimpel 2016). To determine the exact mechanism of *M. sacchari* mortality, or lack thereof, by parasitoid attacks future studies will be needed.

Parasitoid size is a strong proxy for overall fitness, with larger parasitoids producing more eggs that are larger and increased adult longevity (Visser 1994; He and Wang 2006). F1 parasitoids were smaller than parental across all species, which suggests *M. sacchari* is possibly smaller than the host that the parental generation was reared in, resulting in smaller offspring (Silava et al. 2011; Jones et al. 2015). Another, and not exclusive possibility is that *M. sacchari* is a lower quality host than the natal host of parental, which would concur with the increased development time of F2 relative to F1 (Silava et al. 2011; Velasco-Hernández et al. 2017). During sorting of parasitoids for the initiation of F1 host acceptance trials, more wasps had to be sorted to find the needed number of females than during the trials for the parental generation. While parasitoids collected for head capsule measurements were not sexed, a possible skew toward smaller male parasitoids could explain the decrease in mean head size in F1. F2 *A. colemani*, however, were significantly larger than F1, indicating a possible increase in suitability of *M. sacchari* as a host for *A. colemani* over multiple generations. Increases in acceptance and suitability of a host may require two to 40 generations to be detected (Pennacchio et al. 1994; Henry et al. 2008).

Although F1 *A. colemani* and *A. ervi* produced more mummies than their parents, the proportion of mummies that successfully developed into adults was lower than their parents (Table 2). *Aphidius* mummies are normally tan. Tan and black mummies were found in both generations. Black mummies were presumed to be larvae that failed to complete development. No emergence holes were found in black mummies and dissections found nothing recognizable as a developing parasitoid. Across all species, 14 ± 3% of mummies produced by parental parasitoids were black. However, 54 ± 3% of mummies produced by F1 were black. The cause of the mummies to fail and turn black is unknown. F1 were smaller than parental, indicating a possible decrease in fitness between generations (Visser 1994).

This study determined that *A. colemani,* and to a lesser extent *A. ervi,* were well suited to use *M. sacchari* as a host and greatly suppressed population growth. On the final day of *M. sacchari* counts in the parental generation, *A. colemani* and *A. ervi* had 75% and 63% fewer in *M. sacchari* relative to controls. In our experiments *A. colemani* produced the most adults and successfully reduced *M. sacchari* population growth. However, the second generation, despite producing as many or more mummies than the parental, failed to suppress aphid population growth. Augmentative field releases of mass-reared parasitoids have mixed results (Höller and Haardt 1993; Montoya et al. 2000; Gardner et al. 2011; Gariepy et al. 2015). However, *A. colemani* has already been found to successfully attack *M. sacchari* in the field in Mexico (Salas-Araiza et al. 2017). Thus, *A. colemani* is a strong candidate for *M. sacchari* management using inundative releases which do not rely on further generations to suppress *M. sacchari* populations. Further testing is needed to determine the effectiveness of field releases of mass-reared aphid *A. colemani* to manage of *M. sacchari*.

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Compliance with ethical standards

Conflict of interests The authors have no conflicts of interest with this work.

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