Parasitism of *Melanaphis sacchari* (Hemiptera: Aphididae) by *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) in the Greenhouse and Field[1]

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Abstract The sugarcane aphid, *Melanaphis sacchari* (Zehntner), is an economically important invasive insect pest of sorghum, *Sorghum bicolor* (L.) Moench, production in the southern United States. Studies were conducted to: (1) assess the fecundity of *Lysiphlebus testaceipes* (Cresson) parasitizing *M. sacchari* in the greenhouse, and (2) examine the temporal synchrony between *L. testaceipes* and *M. sacchari* in the field during 2017 and 2018. Results indicate that after 96 h, the number of *M. sacchari* increased approximately 90-fold from a single adult in the greenhouse study. The percentage of mummification observed in the greenhouse study was 15.2 ± 3.3%. Of those mummies, *L. testaceipes* adults emerged from 88.7 ± 4.4%, with 38.6 ± 8.4% of those males. Field observations showed that *L. testaceipes* appeared in the sentinel fields in late July to August. The percentage of mummies observed in the June- and July-planted plots of 2017, and June-planted plots of 2018 were $4.5 \times 10^{-4} \pm 1.5 \times 10^{-4}$%, $7.4 \times 10^{-4} \pm 4.4 \times 10^{-4}$%, and $4.4 \times 10^{-5} \pm 1.7 \times 10^{-5}$%, respectively. Even though parasitization of *M. sacchari* by *L. testaceipes* was observed in both field and greenhouse, the lack of wasps before late July and low rate of parasitization in the field suggest that this species is not well synchronized with *M. sacchari* populations. Additional natural enemies (e.g., other parasitoids, predators, and entomopathogens) or other adaptations will be necessary before this pest is managed using biological control in Georgia.

Key Words biological control, parasitoid, grain sorghum, population dynamics

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), is a recent invasive pest of sorghum, *Sorghum bicolor* (L.) Moench (Poaceae), production in North America. Several species of grasses (Poaceae) have been recorded as hosts of *M. sacchari* (Singh et al. 2004). For example, *M. sacchari* is an economic pest of sorghum in Australia, Asia, Africa, and South America (Singh et al. 2004). *Melanaphis sacchari* was first reported in the United States from Hawaii in 1896 (Pemberton 1948), followed by reports from Belle Glade, FL, in 1977 from

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sugarcane, *Saccharum officinarum* L. (Mead 1978), and later in Louisiana in 1999 (White et al. 2001). Denmark (1988) documented sugarcane aphids feeding on sorghum in Florida, but did not report economic losses. The first significant infestation of *M. sacchari* on grain sorghum in the United States was reported along the Texas Gulf Coast and Louisiana (Villanueva et al. 2014). The insect was first detected in Georgia in August 2014 and had spread to South Carolina by mid-September of the same year (G.D.B. unpubl.). By the end of 2015, *M. sacchari* was reported from 17 states and Mexico feeding on grain sorghum, sorghum–sudan hybrids (*S. bicolor* × *S. bicolor* var. Sudanese), sweet sorghum, several millet varieties (*Panicum miliaceum* L.), and johnsongrass, *Sorghum halepense* (L.) Persoon (Bowling et al. 2015). Although *M. sacchari* can be found on field edges in corn, *Zea mays* (L.) (Poaceae), and cotton, *Gossypium* spp. (Malvaceae), these are nonreproductive hosts. The *M. sacchari* genotype specializing on sorghum likely resulted from transportation of the alates to North America by wind or through human activities (Bowling et al. 2016). This genotype, designated as MLL-F, belongs to one asexual clonal lineage that is believed to have originated either from Africa or Asia, the latter being the most probable (Bowling et al. 2016, Harris-Shultz et al. 2017, Nibouche et al. 2018).

Melanaphis sacchari is most commonly found on the abaxial side of sorghum leaves, where they establish and feed, avoiding direct sunlight. Like other types of aphids, *M. sacchari* is a phloem feeder (Singh et al. 2004). Plant damage occurs due to direct loss of plant nutrients, leaf chlorosis, and lost photosynthetic ability following development of secondary sooty mold that occur on honeydew excreted by the aphids. A loss of $62 to $432 per hectare has been reported from fields with a pest density of 250 aphids per leaf (Bowling et al. 2016). Yield loss is attributed to poor plant vigor and head emergence, in addition to inefficient harvest due to high aphid density and sticky honeydew (Villanueva et al. 2014). In the United States, *M. sacchari* propagates through asexual reproduction only. Overwintering adults and nymphs survive on remnant and ratooning sorghum as well as johnsongrass in the southern United States (Bowling et al. 2016).

A number of *M. sacchari* natural enemies are reported in studies from Texas. These beneficial insects include a number of aphidophagous predators and *Aphelinus* sp. nr. *varipes* (Hymenoptera: Aphelinidae) (Bowling et al. 2016, Colares et al. 2015a). Compared with a suite of generalist predators, coevolved parasitoid specialists often have the strongest impact in suppressing pest populations (Schmidt et al. 2003, Snyder and Ives 2003, Stiling and Cornelissen 2005). Therefore, identification and conservation of a key parasitoid attacking *M. sacchari* could lead to improved and effective biocontrol programs.

Because *M. sacchari* is a novel host for the aphid parasitoids in Georgia, any information about the associations between these species and the rates of parasitization is limited. In Kansas and Texas, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) was reported to attack *M. sacchari* in sorghum fields (Colares et al. 2015b, Knutson et al. 2016). Objectives of the study reported herein were to: (1) assess the fecundity of *L. testaceipes* parasitizing *M. sacchari* in the greenhouse, and (2) examine the temporal synchrony between *L. testaceipes* and *M. sacchari* in the field during 2017 and 2018.

Materials and Methods

Greenhouse study. A continuous supply of grain sorghum hybrid plants was maintained in the greenhouse to support the research. Three sorghum seeds (SS-800A, Southern States Coop. Inc., Richmond, VA) were sown per 11.4-liter plastic pots filled with Metro Mix 300 growing medium (Sun Gro Horticulture, Bellevue, WA) and fertilized bimonthly with 6.5 g of Osmocote 14-14-14 and 5.5 g of Micromax 90505 (The Scotts Co. LLC, Marysville, OH). This sorghum hybrid was selected due to its known susceptibility to *M. sacchari*. At the 2-leaf stage, two of the three seedlings were culled leaving one plant per pot. The potted sorghum plants were watered daily using automated sprinklers that provided 400 ml of water per pot.

A laboratory colony of *M. sacchari* was initiated from a natural infestation (approximately 300 adults) occurring on grain sorghum from Tift Co., GA, in October 2016. The colony was maintained in the greenhouse during the winter months on sorghum plants (starting with the 7-leaf stage) under a 13:11 (L:D) photoperiod and temperatures that fluctuated between 20 and 25°C and 60 ± 10% relative humidity (RH).

The greenhouse colony of *M. sacchari* was found to be parasitized during March 2017 (S.L. pers. obs.) (Fig. 1). The resulting aphid mummies were collected and held until the parasitoids emerged. Those adults were then identified as *L. testaceipes* using a taxonomic key (Pike et al. 1997) followed by a confirmation by N.C. Elliot (USDA-ARS, Stillwater, OK); voucher specimens have been deposited in the University of Georgia Collection of Arthropods in Athens. A subsequent parasitoid colony was maintained on live sorghum plants infested with *M. sacchari* inside a fine mesh cage (model BD2120, MegaView Science Co., Ltd., Taichung, Taiwan).

Experimental exclusion cages were fabricated from polyethylene pipe insulation with wall thickness of 1 cm and internal diameter of 3 cm (Therma-Cel®, Nomaco Insulation, Tarboro, NC). The pipe insulation was cut into 2-cm-thick rings using a band saw and then the opening on one side of the ring was covered with white organza fabric (Jo Ann Stores LLC, Hudson, OH) adhered with hot-melt glue. With the fabric-covered ends distal to the leaf surface, two sections of pipe insulation were tightly sandwiched over a sorghum leaf using hook-and-loop tape (Velcro USA Inc., Manchester, NH) that was attached to the pipe insulation using hot-melt glue. Small amounts of cotton lint were forced into any minute gaps between the cage and the leaf to prevent confounding emigration or immigration.

After the potted sorghum plants reached the 8-leaf stage of vegetative growth, the uppermost fully expanded leaf was selected for experiments. A single apterous adult was gently transferred from the aphid colony to the abaxial leaf surface using a fine bristle brush (number 0, Yangzhou Fuxin Artist Brushes Co., Ltd., Jiangsu, China), and the exclusion cage was fastened over the aphid to restrict the aphid from escaping (Fig. 2). The aphid was then allowed to feed and produce offspring. After 96 h, the leaf disk surrounded by the aphid cage with caged aphids was carefully cut from the plant and placed in an 8-cm-diameter polystyrene petri dish (P5856, Sigma-Aldrich, Milwaukee, WI) lined with dry filter paper (Fisherbrand® Filter Paper P8-creped, Fisher Scientific, Pittsburgh, PA). Immediately thereafter,

Fig. 1. ***Melanaphis sacchari*** **aphids and natural enemies on a sorghum leaf. Note** ***M. sacchari*** **mummies (tan in color), formed after parasitization by** ***L. testaceipes,*** **are present on the left side of the image, a dusky lady beetle larva,** ***Scymnus*** **spp. (Coleoptera: Coccinellidae), is located near the center, while** ***L. testaceipes*** **adults are present at the top of the leaf and directly to the left of the dusky lady beetle larva.**

digital images of each leaf disk were acquired in order to accurately discern quantity and life stage of the individual aphids.

Next, a single female *L. testaceipes* (24 h old) was introduced into the petri dish for 24 h, following the methods of Colinet et al. (2005). During this time the petri dishes were held in an environmental growth chamber maintained at 25 ± 1°C, 85 ± 1% RH, and on a 14:10 (L:D) photoperiod. Prior to the onset of each experiment, each freshly eclosed female *L. testaceipes* was held in a test tube provisioned with honey to generate parasitoids of known age and mating status (Lahiri et al. 2017). Two males per female parasitoid were present in each test tube for 4 h to ensure mating as Persad and Hoy (2003) observed *L. testaceipes* mating within 1 h of confinement. At the end of 24 h, the parasitoids in the petri dishes were removed using an aspirator and the leaf disk, along with aphids, was carefully transferred onto the organza fabric of the exclusion cage and the cage was attached to the abaxial side of a new leaf that was attached to a potted sorghum plant growing in the growth chamber. The aphids were held in the cage for the development of aphid mummies, which are tan in color. After 6 d, aphid mummies were collected with a fine brush and stored in a test tube held in the growth chamber until emergence.

Fig. 2. Exclusion cage confining *M. sacchari* to a grain sorghum leaf during the greenhouse study.

The percentages of aphid mummies and emerging parasitoids were scored relative to available *M. sacchari* hosts. A total of 10 replicates of this study were initiated on 23 March 2017 and 10 additional replicates were initiated on 12 April 2017. A total of 5 replicates had to be discarded due to parasitoids or predators gaining access to the exclusion cages during the experiment and confounding the results, leaving a total of 15 replicates for analyses.

Field survey. Field survey of *M. sacchari* and *L. testaceipes* occurrence in sentinel grain sorghum plots were performed in 2017 and 2018. In 2017, three irrigated plots (45.7 m × 16 rows with a 91.4-cm row spacing) were planted with a susceptible grain sorghum hybrid (SS-800A), located at the University of Georgia Lang-Rigdon Farm (N 31.5170, W −83.5483). Each of these three plots were planted on three different planting dates. Planting dates included 10 May 2017 (designated as May_Sorghum), 2 June 2017 (June_Sorghum), and 7 July 2017

(July_Sorghum). Seeds were not treated with any fungicides or insecticides and the fields remained unsprayed throughout the season. In 2018, a single planting date (6 June 2018) with a susceptible grain sorghum hybrid (DKS53-53, Bayer Corp, Whippany, NJ) was planted at the same location with a similar plot size. Each plot (45.7 m × 16 rows with a 91.4-cm row spacing) was observed for arrival of both *M. sacchari* and *L. testaceipes*. To facilitate this field sampling, 10 randomly selected sorghum plants per plot were sampled weekly where the ratio of tan mummies to healthy *M. sacchari* present on the bottom green leaf of each plant was recorded. During weekly sampling, the growth stage of the sorghum plants was assessed following the methods of Vanderlip and Reeves (1970).

Data analysis. Mean and standard error were calculated for responses, including *M. sacchari* progeny and *L. testaceipes* percent parasitization, male progeny proportion, and percent mummy hatch. Percent parasitization of *M. sacchari* was calculated as the number of mummies divided by the sum of apterous adults plus third and fourth instars. Alates were excluded from this calculation because they rarely formed mummies and, if they did, they very rarely produced adult parasitoids. Male progeny proportion of *L. testaceipes* was calculated as male progeny divided by total progeny. *Lysiphlebus testaceipes* percent emergence was calculated as the total *L. testaceipes* adults that emerged divided by total number of mummies formed in each replicate.

In the field study, pest and parasitoid abundance and percent parasitism were calculated for weekly observations in sentinel plots. Additionally, the presence and abundance of *L. testaceipes* and aphid mummies were recorded. Summary data were plotted to show temporal occurrence of the species in the field.

Results

Greenhouse study. Sugarcane aphids produced many offspring in the greenhouse. After 96 h, a mean of 93.3 ± 10.3 ($n = 15$) sugarcane aphid offspring (all life stages) developed in each exclusion cage. Fewer than 10 individuals per replicate completely molted to the adult life stage, as the majority of the individuals developed to 2nd and 3rd instars (Fig. 3). *Lysiphlebus testaceipes* parasitized *M. sacchari* at an average rate of 15.2 ± 3.3%. Adult parasitoids emerged from the majority of the mummies (88.7 ± 4.4%), with 38.6 ± 8.4% of the progeny being male.

Field observation in sentinel plots. Although *L. testaceipes* were observed in the field, the percent parasitism was extremely low in both years. In 2017, *M. sacchari* alates initially appeared in the May_Sorghum plot on 30 May 2017, but *L. testaceipes* were never observed in that plot. In the June_Sorghum plot, *M. sacchari* alates were first observed on 13 June, while *L. testaceipes* were first observed 7 weeks later on 8 August (Fig. 4). The overall percent parasitism by *L. testaceipes* in this plot was found to be $4.5 \times 10^{-4} \pm 1.5 \times 10^{-4}\%$. In the July_ Sorghum plots, *M. sacchari* alates were first observed on 15 August, and *L. testaceipes* were first observed on 22 August. The overall percent parasitism by *L. testaceipes* in this plot was found to be $7.4 \times 10^{-4} \pm 4.4 \times 10^{-4}\%$. Due to overwhelming *M. sacchari* pressure, none of the plants in the May_Sorghum plot

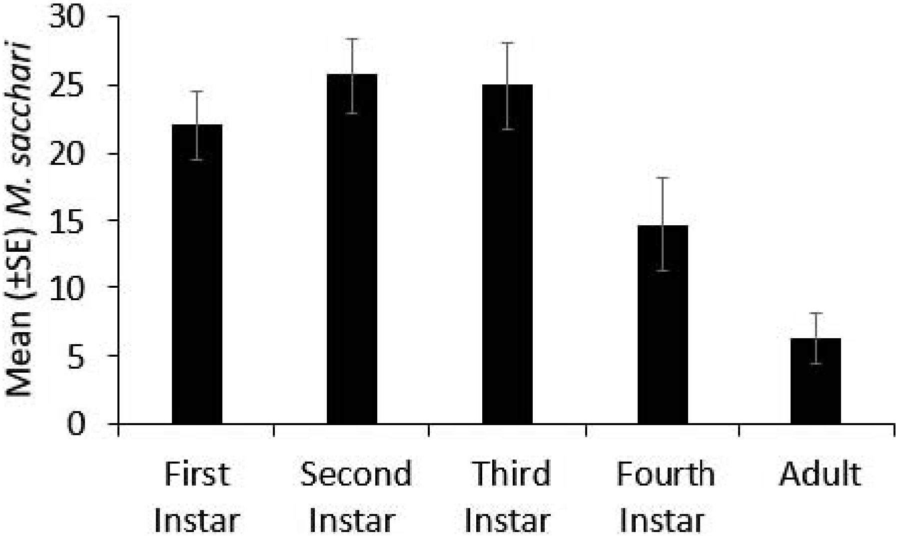

Fig. 3. Mean (± SE) number of *M. sacchari* immature and adult individuals 96 h after caging a single apterous adult under greenhouse conditions ($n = 15$).

progressed beyond the boot stage. However, plants reached maturity and produced grain in the two later planting dates.

In 2018, *M. sacchari* alates were first detected on 27 June, but *L. testaceipes* was not observed until 5 weeks later on 25 July. The overall mummy percentage in the June planted sentinel plot was recorded at $4.4 \times 10^{-5} \pm 1.7 \times 10^{-5}$%.

Although it was not the target pest species in this study, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) was observed infesting the sorghum panicles and feeding on the flag leaf during the time that *L. testaceipes* started appearing in August 2017. Within 1 week of *R. maidis* infestation, *L. testaceipes* parasitized nearly 100% of *R. maidis*. One week after first observation, nearly all *R. maidis* were mummified, leading to a *R. maidis* population collapse (S.L. pers. obs.). To confirm the natural enemy that parasitized *R. maidis*, the mummies were collected from the field and held in a growth chamber until the adults emerged. Those adults were then keyed to *L. testaceipes*.

Discussion

The aphid parasitoid *L. testaceipes* was found to parasitize *M. sacchari* under both greenhouse and field conditions in Georgia. However, the rate of parasitization in the greenhouse assay was much greater than the very limited parasitism observed in the field. The fecundity of *M. sacchari*, under the present experimental conditions, was high (approximately 90 aphids in 4 d). The highest daily birth average of *M. sacchari* on artificial diet (30% sucrose + 0.5% yeast) during the first 4

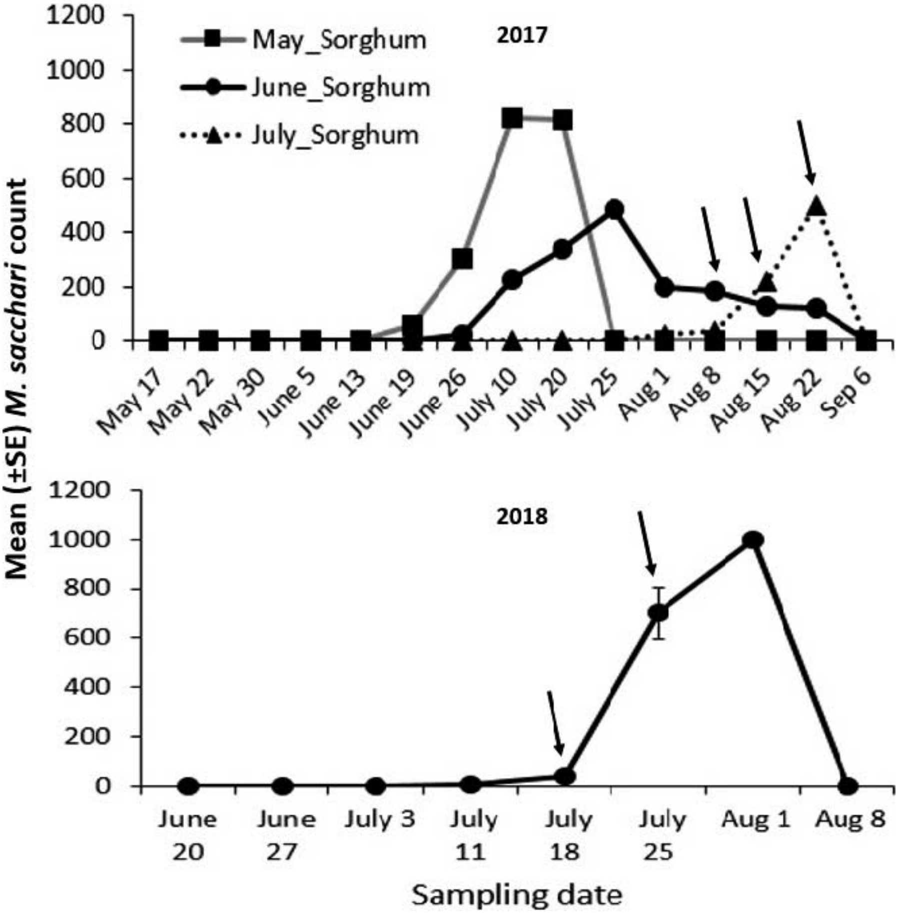

Fig. 4. Mean (± SE) number of *M. sacchari* in sentinel grain sorghum plots planted on 10 May 2017, 2 June 2017, and 7 July 2017 (top) and on 6 June 2018 (bottom). Presence of tan mummies resulting from *L. testaceipes* parasitization are shown with arrows.

d was reported as 28.9 ± 10.3 (Toledo-Hernandez et al. 2018). Access to a live host caged under optimal environmental conditions may explain the *M. sacchari* population explosion in the present study. *Lysiphlebus testaceipes* is capable of rapid host range expansion, as evident by its role as an exotic aphid parasitoid along the West Mediterranean and Atlantic coastal areas of southern France, Spain, and Portugal (Starý et al. 2004). Hosts of *L. testaceipes* in North America include *R. maidis* (Vinson and Scarborough 1991); bird-cherry oat aphid, *Rhopalosiphum padi* (L.); Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Pike et al. 1997); brown citrus aphid, *Toxoptera citricida* Kirkaldy (Persad and Hoy 2003); and greenbug, *Schizaphis graminum* (Rondani) (Starks and Burton 1977).

Therefore, the current host range of *L. testaceipes* in the United States already includes exotic invasive aphid species to which it has adapted to parasitize. However, rates of parasitism by *L. testaceipes* can vary widely by host aphid. For example, *L. testaceipes* parasitized 67% and 76% of *S. graminum* and 46% and 56% parasitism of *Aphis gossypii* Glover, under choice and no-choice test conditions in the laboratory (Rodrigues and Bueno 2001). *Lysiphlebus testaceipes* parasitized 86% of *T. citricida* in the field, despite the presence of red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Interestingly, only 26% parasitization was observed in a laboratory study in the presence of *Solenopsis invicta*, due to preferential predation of parasitized aphids (Persad and Hoy 2003). Vinson and Scarborough (1991) documented that *Solenopsis invicta* selectively destroyed *R. maidis* mummies that were parasitized by *L. testaceipes*. After comparing performance of *L. testaceipes* on three aphid species, *Aphis craccivora* Koch, *Aphis fabae* Scopoli, and *Aphis gossypii*, at temperatures of 17, 22, and 27°C, *A. fabae* was found to be the ideal host at 20–22°C (Satar et al. 2018).

In this field study, *L. testaceipes* parasitized virtually every *R. maidis* present as soon as they appeared. Temporally, both *R. maidis* and *L. testaceipes* appeared in early August and *L. testaceipes* seemed to be spatially and temporally synchronized with *R. maidis*. Conversely, *M. sacchari* and *L. testaceipes* were also present during late July and throughout August, but the results were very different. One difference between the two aphids is that *R. maidis* and *M. sacchari* spatially occupy different zones of the plant. Feeding on the upper foliage, including the whorl and flag leaf, is typical of *R. maidis* infestation in corn and sorghum (Foott 1977, Li et al. 2008, Park and Obrycki 2004). Conversely, *M. sacchari* infestations start at the bottom of the plant and gradually move upward. A second difference is that *M. sacchari* infestations started much earlier in the year than *R. maidis*. Jackson et al. (1970) documented that *L. testaceipes* activity in the field in Oklahoma was not evident until August. In Georgia, the authors never observed *L. testaceipes* in the field until late July to August.

Although *L. testaceipes* does not seem to be a strong candidate for biological control of *M. sacchari* in Georgia at this time, there are other candidate species that need to be evaluated. Preliminary work in Georgia in 2017–2018 suggests that *Aphelinus nigritus* Howard is also present at multiple locations and could be an important parasitoid of *M. sacchari* (G.D.B. unpubl.). Additionally, there may be other native parasitoids that can adapt to *M. sacchari* over time.

Melanaphis sacchari is a highly fecund species, even compared with other aphids (Bayoumy et al. 2016). Future studies are required to elucidate new natural enemies or better adapted native species to serve as effective biological control agents in integrated pest management programs.

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