HELPERS AND HINDRANCES:

THE ROLE OF ECOLOGICAL FACTORS MEDIATING FUTURE BIOLOGICAL CONTROL OF SORGHUM APHIDS

A Dissertation

by

CRYSTAL WRIGHT

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Raul F. Medina
Co-Chair of Committee,	Keyan Zhu-Salzman
Committee Members,	Julio S. Bernal
	Ronnie W. Schnell
	Suhas Vyavhare
Head of Department,	Phillip Kaufman

August 2021

Major Subject: Entomology

ABSTRACT

Aphids are among the most damaging pests of cereal crops. Because aphid control is largely chemical, little attention has been given to ecological complexities that promote aphids' success. How aphids interact with antagonists (natural enemies) and potential mutualists (ants) is important because these interactions can impact the feasibility of biological pest control. One recent and invasive pest of grain sorghum, *Sorghum bicolor*, is the sorghum aphid, *Melanaphis sorghi* (SA). Understanding this pest's ecology in the grain sorghum agroecosystem is critical to developing SA control strategies. Consequently, this dissertation studied SA interactions with a common parasitoid (*Aphelinus nigritus*) and a potential ant mutualist (the red imported fire ant *Solenopsis invicta* (RIFA)) to assess the practicality of SA biocontrol. Since parasitoids often use aphid honeydew as a sugar resource, SA honeydew was first assessed as a potential attractant to *A. nigritus*. As SA feeds on grain sorghum and the nearby overwintering host Johnson grass, *Sorghum halepense, A. nigritus* preference for SA honeydew produced on either host plant was also assessed. Ultimately, *A. nigritus* was attracted to SA honeydew and preferred honeydew produced on Johnson grass, which could support the augmentation of this parasitoid in Johnson grass to suppress SAs before grain sorghum is planted. Second, a potential for SA to exhibit fecundity compensation (i.e., a rapid increase in reproduction in response to natural enemies) was explored to determine whether SAs could defend themselves by increasing their fecundity after experiencing wounding akin to parasitoid oviposition. Fecundity compensation was observed in daughters of aphid mothers parasitized by *A. nigritus*, which may question the use of certain parasitoids in SA biocontrol programs. Third, the effects of RIFAs on SA population growth were assessed over two field seasons. It was determined that RIFA increases SA

populations, but only when initial aphid densities are low. This result provides baseline data on potential mutualistic interactions between two invasive species, SA and RIFA, allowing future monitoring of its evolution. As a relatively new invader, the 2013 arrival of SA grants a unique opportunity to explore aphid ecological adaptations to new environments, an often-overlooked factor that may inform future biocontrol.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Medina, co-chair, Dr. Zhu-Salzman and my committee members, Dr. Bernal, Dr. Schnell, and Dr. Vyavhare, for their guidance and support throughout the course of this research. I want to thank my siblings for listening to my research-related stories, even though they vehemently detest insects. Their dedicated ears and sense of humor have made my graduate experience fun, even on the hard days. Thanks also go to my parents, who made the 19-hour sacrifice to drive me and my belongings to College Station all those years ago. I also want to acknowledge my extended family of aunts, uncles, and my grandmother who is still active at 80 years of age. They inspire me to live a fulfilling life and not worry about any issues or setbacks.

My colleagues and friends in the Department of Entomology are a fun group. Through interactions with many different people, I've learned to enjoy my PhD, and not see it simply as a career necessity. I also appreciate this college town for giving me a different perspective on higher education and sense of community. Lastly, I am happy and lucky to have lived with strong, opinionated, and entertaining roommates these past five years. It was always nice coming home to these people, and I hope I meet others like them in life.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professor Raul F. Medina (advisor), Professor Keyan Zhu–Salzman (Co-advisor) and Professors Julio S. Bernal and Suhas Vyavhare of the Department of Entomology and Professor Ronnie W. Schnell of the Department of Soil and Crop Sciences.

Experimental design and training in High Performance Liquid Chromatography was provided by Dr. Anjel Helms in the Department of Entomology. Data analysis for Chapter 2 was conducted in part by John Grunseich. Field sites and farming equipment for Chapter 4 were provided in part by Al Nelson, Pete Krauter, and Cesar Valencia. Identifications of specimens collected from field sites were provided by Erin Maxson and Professor James Woolley in the Department of Entomology. All other work conducted for the dissertation was completed by the student independently.

Funding Sources

Graduate study was supported by a fellowship from Texas A&M University's Program in Exemplary Mentoring and teaching assistantship from the Department of Entomology. This study was also supported by the Dr. Roger E. Gold Endowed Graduate Scholarship from the Department of Entomology and research assistantship from the Oliva Chavez lab.

This work was further made possible in part by the Southern IPM Center under grant number 2018-3200-25 and the Invasive Ant Research and Management Project under grant number

2018-70006-28884. Contents of both grants are solely the responsibility of the authors and do not necessarily represent the official views of the Southern IPM Center or Texas A&M AgriLife.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Aphids are prolific pests of cereal crops. As phloem feeders, they strip crops of key sugars, nutrients, and water, and ultimately hinder plant growth and development. Since aphids reproduce parthenogenetically, their populations grow exponentially in short periods of time. As a result, aphid feeding can rapidly damage and reduce crop yields [1]. Many aphid species are competent vectors of plant viruses, which can accelerate declining crop health [2]. To mitigate pest damage, pest management practices rely heavily on chemical control, which although effective, often leads to insecticide resistance [3-7], as well as human health and environmental risks [8-14]. As a result, a growing number of farmers have adopted integrated pest management (IPM), a broad-based strategy that relies on synergistic combinations of chemical, cultural, and biological control practices [15]. Adoption of IPM has mitigated pest damage in while reducing chemical use in several cropping systems [16-18].

IPM often involves biological control. Biological control (a.k.a., biocontrol) refers to the regulation of a pest by natural enemies (i.e., predators, parasitoids, and pathogens) [19]. As such, it is a practice that examines the interspecific interactions between pests and other species [20]. This management practice can effectively reduce pest damage, and under ideal conditions it may reduce pest populations below economic threshold levels while significantly reducing chemical applications [21-23].

Biological control has been effective in reducing aphid populations and other hemipteran pests on several agricultural crops [22, 24-29]. For example, the parasitoid *Trioxys pallidus*

(Hymenoptera: Aphidiidae) effectively suppresses aphid populations of *Panaphis juglandis* and *Chromaphis juglandicola* on Californian walnuts [30, 31]. Similarly, the introduced parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) has reduced aphid populations of *Brachycorynella asparagi* and *Diuraphis noxia* on asparagus and mustard croplands in Washington [32]. Predators can also suppress aphid populations on crops; for example, the augmentation of coccinellids on Arkansas cotton has successfully lowered populations of *Schizaphis graminum* and *Diuraphis noxia* [27]. Nonetheless, most control agents are parasitoids because they tend to have more specialized host ranges, and consequently, fewer non-target effects can be expected, compared to predators [33, 34].

Any successful biological control program must consider ecological factors that influence the impacts of natural enemies. Typically, these factors include: phenological synchrony with pests [35], natural enemy and pest abundances, and potential effects of natural enemies on non-target organisms, among others [36]. That said, commonly overlooked factors include the attraction to pest by-products, pest defenses, and pest mutualists, which may alter the dynamics of natural enemy-pest interactions. These factors are important, as it can help [37] or hinder [38] the ability of some natural enemies to exert effective pest control.

The sorghum aphid: An Economically Important Pest of Grain Sorghum

A cereal pest of economic concern in Texas is the sorghum aphid (hereafter SA), *Melanaphis sorghi* (Theobald, 1904) (Hemiptera: Aphididae). SA was originally reported as the sugarcane aphid, *Melanaphis sacchari* (Zehntner, 1897), and has become a serious pest of grain sorghum, *Sorghum bicolor* [39]. SA is mostly an anholocylic species in North America, wherein nymphs

are born alive (viviparously) and produced through parthenogenesis [40]. The only sexual egg-producing (oviparous) reports of SA are from Mexico in the states of Guanajuato, Querétaro, and Sinaloa [41]. Nymphs can become adults in as short as 5 days [42]. In total, the SA lifecycle ranges from 10-37 days [43]. Morphologically, SA adults are either alate (winged) or apterous (wingless), and both forms have gray, tan, or light-yellow bodies. Adults and nymphs have dark tarsi, cornicles and antennae. Summer forms tend to be light in color, while winter forms range in colored appearance from gray to dark yellow [44]. Adult alate aphids often have black markings on the dorsal sclerites, and always have black wing veins [45, 46].

Following initial 2013 reports along the Texas Gulf Coast, the rapid expansion of SA in the US has raised serious concerns, as the pest has reduced sorghum yields and caused economic loss [47, 48]. Feeding by SA causes chlorosis, delays flowering, and reduces grain quality [49]. Often, the saprophytic fungus *Macrophomina phaseolina* will grow on honeydew secreted by the aphid, which reduces leaf photosynthetic activity [50-52]. In addition to reducing the quality of sorghum grain heads, honeydew secreted by SA can also stick to- and damage harvesting equipment [47]. Economically, the arrival of SA significantly hindered sorghum production in Texas. In the Texas Lower Rio Grande Valley alone, the total loss due to SA from 2013 through 2015 is estimated at $40.95 million, or 19% of the total economic value of sorghum production [53].

Potential for Biological Control in Sorghum Aphid Management

Through SA control efforts today are largely chemical, and range from preventive seed-treatments to post-infestation insecticide sprays [42, 44, 48, 54], the occurrence of a large

number of SA natural enemies [50] make this pest a potential target for biological control. Multiple SA natural enemies, consisting of coccinellids, chrysopids, syrphids, anthocorids, braconids, and aphelinids, are reported on sorghum during its March-September growing season in US southern states [28, 44, 55]. Of these natural enemies, *Aphelinus nigritus* Howard (Hymenoptera: Aphelinidae) is the most commonly reported parasitoid attacking SA in College Station, TX [56]. As parasitoids are the most frequent natural enemies, *A. nigritus* could serve as a model for parasitoid-mediated control of SA. To determine whether SA parasitoids are suitable for biological control, an important first step is to assess how parasitoids find and become attracted to the target host.

Several parasitoid species are known to use honeydew as a kairomone during host searching [57]. Honeydew can also serve as a nutritional source of sugars and amino acids [58]. In some cases, parasitoid preference for honeydew is modulated by the host's diet [59], which may lead to differential parasitism when the same host species feeds on different host plants [60]. Differential parasitism is relevant to biocontrol of SA, given that it is a polyphagous aphid, and feeds on Johnson grass, *Sorghum halepense*, which frequently grows in the vicinity of sorghum. Whether the attractiveness of SA honeydew to parasitoids is mediated by the SA host plants is presently unknown. In chapter II, I explore how honeydew composition (in terms of sugar and amino acid content) and quantity is shaped by the SA host plant, and how this may mediate attraction of the parasitoid *A. nigritus*.

Most aphid biological control focuses on rates of natural enemy suppression. Yet, little attention has been given to the potential for aphid compensation. Some aphids have a range of defense or

compensatory mechanisms that can significantly complicate control efforts [61-63]. These mechanisms may involve the production of repellant chemicals [64-67], rapid escape behaviors [68, 69] or accelerated reproduction [70]. In chapter III, I explore the degree of SA compensation, with specific regard to increased reproduction, against parasitism.

Aphid pests and their natural enemies do not interact in a void, as frequently there are mutualistic species that may mediate pest-natural enemy interactions, and affect natural enemy suppression [20, 71-73]. Aphid mutualisms, particularly with ants, are widespread in nature [74-79], and common in agriculture [71, 80, 81]. In some species of aphids, mutualisms with ants have increased aphid abundance on crops [82]. This usually occurs through ant-mediated protection from natural enemies [71, 83], which reduces aphid mortality and impedes control. As such, the degree of SA suppression by natural enemies may also be modulated by the occurrence and intensity of SA mutualists. Whether or not SA mutualists affect natural enemy-mediated control is an unknown, but relevant, piece of information for SA biological control and sorghum production. This is especially important considering that SA, a recent invader, is already being tended by the aggressive red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae) (RIFA) (J. Holt, unpublished data). In chapter IV, I explore the potential for an SA mutualism with RIFA, and its possible impact on aphid management.

The 2013 arrival of SA granted a unique opportunity to study how aphids adapt to their new environments. Although we know SA, *A. nigritus*, and RIFA all reside in or near grain sorghum and Johnson grass, a comprehensive understanding of the potential impact of their ecological interactions is currently lacking. On one hand, SA could form beneficial relationships with

opportunistic ant tenders seeking a honeydew reward. This same honeydew might attract parasitoids in search of quality hosts and sugar resources, which could then trigger SA compensatory responses. Altogether, assessing each of these factors improves our understanding of SA as a pest and player in the grain sorghum agroecosystem.

CHAPTER II

APHELINUS NIGRITUS HOWARD (HYMENOPTERA: APHELINIDAE) PREFERENCE FOR SORGHUM APHID, *MELANAPHIS SORGHI*, HONEYDEW IS STRONGER IN JOHNSON GRASS, *SORGHUM HALEPENSE* THAN IN GRAIN SORGHUM, *SORGHUM BICOLOR*

Parasitoids locate their hosts using a variety of visual and olfactory cues. In the context of tri-trophic interactions, success in finding hosts is largely influenced by host plant. That is because plants contain physical and chemical characteristics that can attract or repel foraging parasitoids [84]. Host plant can alter parasitoid searching strategy, especially in cases where the same host feeds on multiple plants. Multi-host feeding can lead to differences in the attack rate of parasitoids on one plant versus another [84-87]. Furthermore, this variation in parasitoid presence on different host plants can create enemy-free space, where hosts living on plants least preferred by parasitoids are better protected [88]. Under agroecosystems, the concept of enemy-free space has been studied in phloem-feeding aphids on optimal versus suboptimal plants [89]. Aphids, whose limited mobility make them highly susceptible to parasitism [90-92], produce honeydew, a sugar-rich waste product that can nutritionally supplement parasitoids [57, 93]. Since many aphids feed on multiple host plants, plant-mediated differences in honeydew may affect parasitoid recruitment, as is seen in some hoverfly species [90]. This could provide an opportunity for enemy-free space among aphids occupying suboptimal plant hosts. Overall, the potential for host plant honeydew-mediated protection of aphids against parasitism is understudied and warrants attention.

Honeydew as a Parasitoid Nutritional Source

Many species of parasitoids require sugar-rich diets to meet metabolic needs. In environments rich with floral resources, nectar is a well-studied and highly nutritious source of food [94-96]. Comparatively, fewer studies have assessed the suitability of honeydew, a sugar-rich waste product of aphids, as a food alternative. Honeydew, which comprises carbohydrates, amino acids, and organic acids [97-101], serves as a food source for some parasitoids [58, 102], and predators [103], along with other organisms like ant mutualists [104], inhabiting low-nectar environments. Several studies have documented honeydew feeding in parasitoids from nectar-poor environments [60, 96, 103, 105]. Honeydew utilization is especially common for parasitoids of cereal aphids, even in the presence of nectar sources. For example, Vollhardt, Bianchi [106] note a higher prevalence of honeydew feeding in cereal aphid parasitoids regardless of whether field margins bearing flowers are present. This is likely because parasitoids reduce energy use costs by remaining within a host patch when consuming honeydew instead of actively searching for flowers.

Honeydew as a Kairomone

In addition to a nutritional source, honeydew is considered a kairomone. Kairomones are semiochemicals found on plants, hosts, or host by-products that benefit another species. Aphid by-products like honeydew, along with glandular [107] and cornicle secretions [65] are key determinants of parasitoid attraction [37] that increase the efficiency of host location and can improve the likelihood of parasitism [57, 93, 108]. In certain cases, strong levels of honeydew kairomones can elicit parasitoid behaviors in the absence of aphids and other hemipterans. For example, *Psyllaephagus pistaciae* parasitoids increase their searching behavior in the presence of

psyllid honeydew alone [109]. As honeydew can lure parasitoids to infested plants and provide an important food resource, the role of honeydew in parasitoid-mediated aphid suppression merits attention.

Honeydew Relevance to Parasitoid Preference in Agroecosystems

Multiple honeydew-producing hemipterans infest agricultural crops, including SA. SA is currently attacked by a variety of predators and parasitoids in the southern US including the generalist parasitoid *Aphelinus nigritus*. *A. nigritus* females are synovigenic and can feed on host hemolymph, honeydew, honey, or plant material to complete egg maturation (Hopper, University of Delaware, pers. communication). When feeding, SAs produce copious amounts of honeydew on sorghum, which may provide a potential food resource to this parasitoid. Since parasitoids can also use honeydew as a contact kairomone, SA honeydew might attract and facilitate *A. nigritus* parasitism. Overall, understanding the degree of *A. nigritus* attraction to SA honeydew will increase knowledge of the dynamics facilitating parasitism in this parasitoid-host system.

Potential for Honeydew Differences in A. nigritus Preference for SAs

While most honeydews comprise a range of sugars, amino acids, and organic acids [60, 76, 110, 111], the composition and concentration of these macronutrients varies among insect hosts, between host plant species, and over time [102, 112]. In the case of SA, it is currently known that SA honeydew varies when feeding on different cultivars of sugarcane, *Saccharum* spp. plant hosts [113]. Honeydew variation is important because it can affect parasitoid preference, as seen with *Aphidius ervi*, which prefers honeydew from the bird cherry-oat aphid, *Rhopalosiphum padi*, over that of the English grain aphid, *Sitobion avenae*, and green peach aphid, *Myzus*

persicae, even when all species feed on the same host plant [60]. In the case of oligophagous or polyphagous insect hosts, parasitoids may prefer honeydew produced by the same aphid species feeding on different plants [114-116]. Honeydew quantity can also influence parasitoid foraging [58], likely because it reflects the population densities of host aphids [106]. In agroecosystems where insects deposit honeydew on both crops and weedy vegetation, differences in honeydew quality and quantity could lead parasitoids to attack insect hosts in one plant over another, resulting in skewed parasitism rates.

This is relevant to SA who feeds on grain sorghum, as well as on the perennial weed Johnson grass, *Sorghum halepense* [117]. This uncultivated weed is abundant across the southern US [118] and frequently occurs in proximity to sorghum. Rates of SA parasitism are reportedly greater on grain sorghum versus Johnson grass (B. Elkins, unpublished data). Furthermore, predation of SA by lady bird beetles and syrphid flies is significantly lower on Johnson grass than on grain sorghum (J. Hewlett, unpublished data). Since host plant largely determines honeydew composition [103, 119-121], differences in SA honeydew chemical composition, along with honeydew quantity, may be driving this natural enemy trend. A better understanding of variations in SA honeydew quality and quantity by host plant and over time may help determine how parasitoids and other natural enemies modulate their selection of hosts.

Consequently, this study assessed both composition and concentration of sugars, amino acids, and organic acids in honeydew from aphids feeding on grain sorghum or Johnson grass. Subsequently, I assayed if aphid host plant diet (i.e., grain sorghum or Johnson grass) and honeydew collection timepoint (i.e., after 24, 72, and 120 hours) mediated honeydew's effect on

A. nigritus parasitoid attraction. As shown in other studies, I predicted a detectible difference in SA honeydew composition between host plants and over time. Considering the trend of less parasitism in Johnson grass, I also predicted a strong *A. nigritus* preference for SA honeydew produced on grain sorghum. Since parasitoid foraging generally increases with larger quantities of honeydew [106, 122], I further predicted a stronger *A. nigritus* response to SA honeydew produced on grain sorghum collected over a period of 120 hours.

Materials and Methods

Establishing Main Aphid Colonies

SAs were reared on either DEKALB® DKS 4420 (Bayer, St. Louis, MO), a susceptible grain sorghum variety or wild Johnson grass collected from the Texas A&M Research Farm in Sommerville, TX (30°31'54.8"N 96°25'50.2"W). Three to five sorghum seeds were planted in 3.8 cm diameter x 21 cm high planting tubes (Amazon, Seattle, WA) containing Sun Gro® Metro-Mix® 360 (Sun Gro® Horticulture, Agawam, MA). The rhizomes of field collected Johnson grass were cut, washed in 2:100 volume of soap and water and replanted in planting tubes. Plants were grown at 27°C under a 16L:8D cycle and 70% relative humidity for three weeks.

Aphids were collected from grain sorghum (30°32'33.0"N 96°25'36.4"W) and Johnson grass (30°32'18.3"N 96°25'04.4"W) field sites and placed on respective grain sorghum or Johnson grass plants in separate 40 x 30 x 30 cm cages to establish main colonies. Main colonies were defined as those containing a genetic mix of SAs. Cages were constructed from plexiglass sheets (ACME Glass Company, Bryan, TX) and fused together with methylene chloride and masking

tape. Grain sorghum and Johnson grass-reared SA colonies were maintained under the conditions listed above. Plants were added to both colonies each week to sustain population numbers.

Establishing Clonal Aphid Colonies

Aphid clonal colony cages were constructed from 1-liter plastic bottles. A 3mm diameter hole was cut around the neck of the bottle, while the bottom portion was completely removed. The base of a plant tube was then fitted through the hole and secured with masking tape. To prevent aphid escape, the bottle was covered with nylon hosiery. With a paintbrush, a single apterous aphid from the main SA colonies generated above was placed on a grain sorghum or Johnson grass leaf in each bottle cage. Aphids were placed on the same plant species from which they were reared in the main colonies. In total, 20 clonal colonies per plant species were maintained in a rearing room under the same conditions mentioned above.

Rearing parasitoids

Grain sorghum or Johnson grass plants infested with SA from the main colonies were placed in separate 40 × 30 × 30 cm cages to rear *A. nigritus*. These *A. nigritus* cages were put in a different room (to avoid parasitoid contamination of the main colonies), under similar light and temperature conditions as the SA main colonies above. *Aphelinus nigritus* mummies (successfully parasitized SA) were obtained from the grain sorghum and Johnson grass field sites referenced above and individually placed in 0.2 ml PCR tubes (Thermo Fisher Scientific, Waltham, MA). Aphid mummies were observed daily until parasitoid emergence. All emerged *A. nigritus* were transferred to *A. nigritus* cages containing grain sorghum or Johnson grass SA-infested plants. Mummies produced in either parasitoid cage were placed in separate PCR tubes

and monitored daily until emergence. Only female parasitoids aged 0 - 24 hours were used in experiments. Once emerged, females were transferred to separate 1.5 ml centrifuge tubes (VWR International, Radnor, PA) containing 2 μL of autoclaved water (smeared on the sides of the tube to allow hydration while preventing drowning) and a 0 - 72 hour-old male. Females and males were observed until mating occurred.

Honeydew Collection

10 apterous, adult aphids from each of the 20 grain sorghum and Johnson grass clonal colonies were transferred via paintbrush to separate clip-cages. Clip cages were lined with round plastic disks for honeydew droplet collection and attached to the leaves of 3-week-old grain sorghum or Johnson grass plants. Aphids were placed on the same plant species from which they were reared. The cages were clipped to each plant and honeydew was deposited continuously for one of 3 timepoints: 24, 72, or 120 hours. After freeze-drying for 24 hours, the honeydew disks were weighed, then diluted in 15μl High Pressure Liquid Chromatography (HPLC)-grade water, filtered through spin columns (Thermo Fisher Scientific) and stored in 1.5ml centrifuge tubes at -20 °C until use. Twenty replicates (clip cages) per plant species and time point were used for HPLC analysis, while 20 other replicates were used in bioassays to assess parasitoid preference.

Analysis of Honeydew Composition Using HPLC

Sugar and organic acid composition of SA honeydew was analyzed through HPLC. Pre-run, samples were thawed and sonicated for 2 minutes, then spun down in a centrifuge at 13,500 rpm. Samples were transferred to 2 ml screw top vials containing 150 μL glass inserts (Agilent Technologies, Santa Clara) and run on the Agilent 1200 binary LC gradient system using the Hi-

Plex Ca (Duo) 7.7 x 50 mm column and 8μL guard column (Agilent Technologies, Santa Clara). This system included a quaternary gradient pump, degasser, and Thermostatted Column Compartment (TCC SL) diode-array detector (DAD) connected to a 1260 Infinity II refractive index detector (RID). The column was eluted with 100% HPLC water at a flow rate of 4.0 ml/min at 80°C. The amino acid composition of SA honeydew was analyzed on the same system using the AdvanceBio AAA 2.7 μm 4.6 x 100mm column with a 2.7 μm guard column (Agilent Technologies, Santa Clara). Two eluent mixtures (A:10mM Na_2HPO_4 – 10mM $Na_2B_4O_7$ at a pH of 8.2 and B: 45:45:10% ACN: MeOH HPLC Grade water) were run through the column at a flow rate of 1.2ml/min at 40°C. Amino acids were derivatized with o-phthalaldehyde (OPA), 9-fluorenylmethyloxycarbonyl (FMOC), a borate buffer, and injection diluent containing 100ml of eluent mixture A and 0.4 ml concentrated H_3PO_4 (Agilent Technologies, Santa Clara) to create fluorescent molecules for enhanced detection at the 390 nm DAD wavelength [123]. Using the Agilent OpenLab ChemStation software, sugar, organic acid, and amino acid identities were determined by comparing retention times to those of authentic standards (Sigma-Aldrich, St. Louis). Quantities for each compound were determined by comparing peak areas to calibration curves.

Statistical analyses were performed using R, Version 4.0.5 (R Core Team, Vienna). Honeydew sugar and amino acid content were analyzed by conducting non-metric multidimensional scaling ordinations in the package VEGAN [124] to visualize blend differences. Permutational multivariate analysis of variance (PERMANOVA) was conducted to quantify differences in blends at different collection timepoints and between host plants [125]. The normality of the data was verified using Levene's Test of Equality of Variances. An ANOVA assessed the effects of

collection timepoint, host plant diet, and an interaction between the two on the total sugar concentration of honeydew. Total sugar concentration of honeydew was the dependent variable, while collection timepoint and host plant diet were independent variables. Tukey's HSD assessed pairwise comparisons in the total sugar concentration of honeydew by host plant and between each collection timepoint. T-tests with Bonferroni corrections were used to compare individual sugar, amino acid, and organic acid compounds between host plants within collection timepoints.

Measuring Parasitoid Preference

Parasitoid preference for either honeydew source was measured at two timepoints: 24 and 120 hours. Five μL of honeydew collected after 24 and 120 hours (from each of the 20 grain sorghum and Johnson grass replicate plants mentioned above) was pipetted onto respective two-week old grain sorghum and Johnson grass leaves. The leaves were then placed on opposite sides of a 30 mm diameter x 11 mm high Petri dish. With a paintbrush, one female parasitoid was placed at the center of the dish. The parasitoid was allowed to acclimate for 5 minutes before recording behavior for 10 minutes using the Behavioral Observation Research Interactive Software (BORIS, Turin, Italy) [126]. Preference was measured as the relative proportion of time spent on each treatment (= total time on one treatment leaf/the total time spent on both treatment leaves). To test parasitoid attraction to either honeydew source, grain sorghum or Johnson grass leaves with a drop of water were used as no-sugar sources in a choice-test setting. The study contained three choice test variations: 1) honeydew produced by SAs feeding on grain sorghum (herein referred to as grain sorghum honeydew) versus water on a grain sorghum leaf, 2) honeydew produced by SAs feeding on Johnson grass (herein referred to as Johnson grass honeydew) versus water on a Johnson grass leaf, and 3) grain sorghum honeydew versus Johnson grass

honeydew on their respective leaves. Accounting for bias toward the original parasitoid aphid host (i.e., whether mothers of experimental parasitoids were reared on grain sorghum or Johnson grass fed SAs), each choice test variation per timepoint consisted of 20 replicates, 10 with parasitoids whose mothers were reared on grain sorghum fed SAs, and the other 10 with parasitoids whose mothers were reared on Johnson grass fed SAs. To avoid directional bias, the locations of plant leaves on the Petri dish were swapped every other replicate. Each replicate consisted of a separate female parasitoid.

Statistical analyses were performed using JMP®, Version 15.2 (SAS Institute Inc., Cary, NC). Data from both honeydew versus water experiments (grain sorghum honeydew versus water and Johnson grass honeydew versus water) at both collection timepoints (24 and 120 hours) were compared to assess the strength of attraction to either honeydew when a second honeydew source was not present. Preference for honeydew as well as by the original parasitoid aphid host and collection timepoint was assessed in an ANCOVA. The relative proportion of time was measured as the total time spent on grain sorghum honeydew (or Johnson grass honeydew)/ the total time spent on each honeydew source and water. The data were converted to arcsine square-root values (ASIN (SQRT x)) × 57.296) and considered as the dependent variable, while collection timepoint, original parasitoid aphid host (i.e., parasitoids whose mothers were reared on grain sorghum or Johnson grass fed SAs) were independent variables. Choice treatment (grain sorghum honeydew, Johnson grass honeydew, or water) was also factored to assess the strength of attraction for one honeydew source over another, between honeydew versus water experiments. Honeydew concentration (dry weight in μg/15μL HPLC-grade water) was used as a covariate to account for notable differences in concentration between 24- and 120-hour samples.

One-sample *t*-tests with Bonferroni corrections were then conducted to compare parasitoid preference within treatments, by collection timepoint, and by original parasitoid aphid host (null hypothesis = no difference in relative time spent on one choice treatment versus another).

Parasitoid Preference: Grain Sorghum Versus Johnson Grass Honeydew

The effect of two available sources of honeydew (grain sorghum and Johnson grass) on parasitoid preference, along with collection timepoint and original parasitoid aphid host was measured in a separate ANCOVA. The relative proportion of time was measured as the total time spent on grain sorghum honeydew/ the total time spent on both grain sorghum and Johnson grass honeydews. As with the honeydew versus water experiments, the data were normalized through arcsine transformations and considered the dependent variable. Collection timepoint and original parasitoid aphid host were independent variables. The difference in concentration between grain sorghum and Johnson grass honeydew was used as a covariate. One-sample t-tests with Bonferroni corrections then compared parasitoid preference within treatments, by collection timepoint, and by original parasitoid aphid host.

Results

SAs Fed on Grain Sorghum and Johnson Excretes Honeydew of Varying Concentrations

The total sugar concentration of honeydew from SAs feeding on grain sorghum and Johnson grass significantly differed by timepoint and by host plant, but there was no significant interaction between the two (Table II-1). Overall, sugar concentration was greater in Johnson grass samples (Figure II-1 A). Total sugar concentrations increased over time although sugar concentration did not vary between the 72 and 120-hour timepoints (Figure II-1 B). Despite this,

there was a trend towards greater concentrations of sugar in Johnson grass versus grain sorghum at each timepoint.

Sugar, Amino Acid, and Organic Acid Profiles Are Similar Between Host Plants

Sugar and organic acid profiles were not significantly different between grain sorghum and Johnson grass honeydew collected after 24 (PERMANOVA F = 3.0852; df = 1,34; P = 0.06) (Figure II-2 A) and 72 (PERMANOVA F = 1.5388; df = 1,34; P = 0.212) hours (Figure II-2 B). There was a significant difference in profiles between honeydews collected after 120 hours (PERMANOVA F = 3.7925; df = 1,34; P = 0.01) (Figure II-2 C). The sugars detected in grain sorghum and Johnson grass honeydew samples are shown in Table II-2. Fructose was marginally more abundant in Johnson grass honeydew than in grain sorghum honeydew after 72 hours (T = 4.215; df = 1,34; P = 0.0474). The only organic acid detected in SA honeydew was fumaric acid, which was more abundant in Johnson grass than in grain sorghum honeydew at both 24 (T = 10.71; df = 1,34; P = 0.0025) and 120-hour (T = 5.325; df = 1,34; P = 0.0272) collection timepoints (Table II-2). Amino acid profiles were not significantly different between host plant honeydews at any of the collection timepoints. Of the detected amino acids, proline, serine, aspartic, and glutamic acid were most abundant on honeydew samples in both plant species. Host plant-specific differences in amino acids were observed for tryptophan after 120 hours (T = 6.04; df = 1,31; P = 0.0198) and Tyrosine after 24 hours (T = 9.401; df = 1,31; P = 0.0041) (Table II-3).

Aphelinus nigritus is Attracted to Honeydew Excreted by Aphids Feeding on Both Grain sorghum and Johnson grass

Across treatments, parasitoid preference (measured as the relative proportion of time spent on each treatment) was not influenced by collection timepoint or by the aphid host from which the parasitoid emerged. In comparing the two experiments, parasitoids preferred honeydew over water, no matter on which host plant honeydew was provided (Table II-4). When given the choice between grain sorghum honeydew and water, both grain sorghum and Johnson grass parasitoids preferred grain sorghum honeydew at both timepoints (Figure II-3 A). Given the choice between Johnson grass honeydew and water, grain sorghum parasitoids preferred Johnson grass honeydew at both 24-hour and 120-hour timepoints. However, Johnson grass parasitoids preferred Johnson grass honeydew at the 24-hour timepoint but made no choice at the 120-hour timepoint (Figure II-3 B).

Aphelinus nigritus Prefers Johnson grass over Grain Sorghum Honeydew

Across treatments, preference was not influenced by collection timepoint, by the aphid host from which the parasitoid emerged, or by an interaction between the two (Table II-5). At the 24-hour timepoint, grain sorghum parasitoids preferred Johnson grass honeydew, while Johnson grass parasitoids preferred grain sorghum honeydew. At 120 hours, both grain sorghum and Johnson grass parasitoids preferred Johnson grass honeydew (Figure II-4).

Table II-1 Results from ANOVA indicate that timepoint and host plant diet affect honeydew total sugar concentration. Bolded values indicate statistical significance at $p < 0.05$.

Variables	F value	df	P value
Collection Timepoint	11.2749	2,104	**< 0.0001**
Host Plant	6.0142	2,104	**0.0159**
Collection Timepoint x Host Plant	0.4947	2,104	0.6112

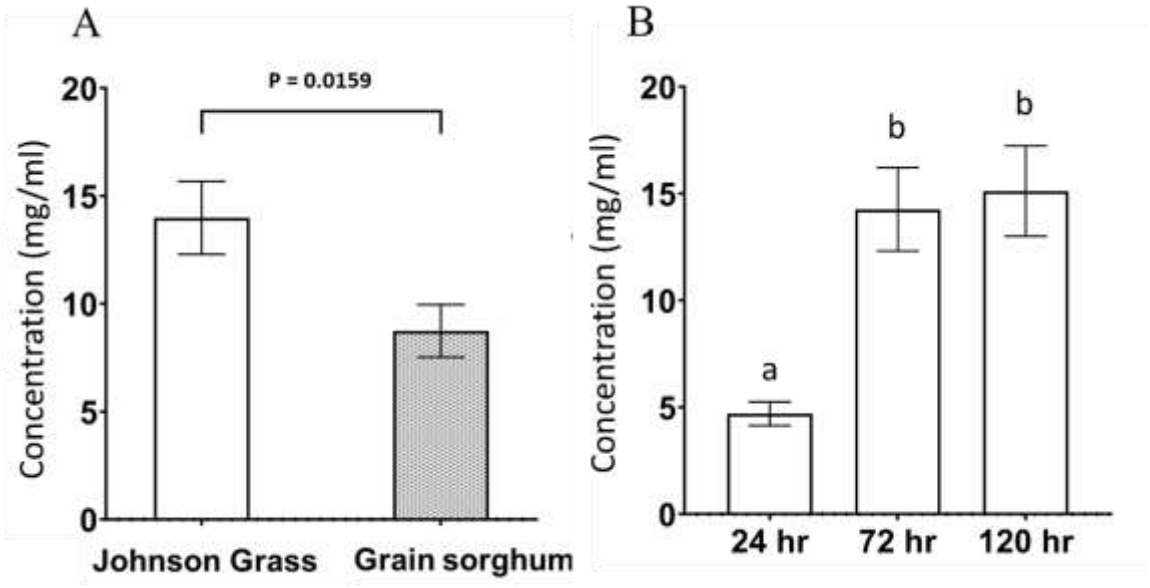


Figure II-1 (A) Overall, sugar concentration of honeydew is greater in honeydew from Johnson grass aphids (see Table I-1). Statistical significance at $p < 0.05$). (B) Total sugar concentrations of SA honeydew increase over time but are indistinguishable between the 72 and 120-hr timepoints. As per Tukey's HSD, different letters indicate statistical significance across timepoints at $p < 0.05$.

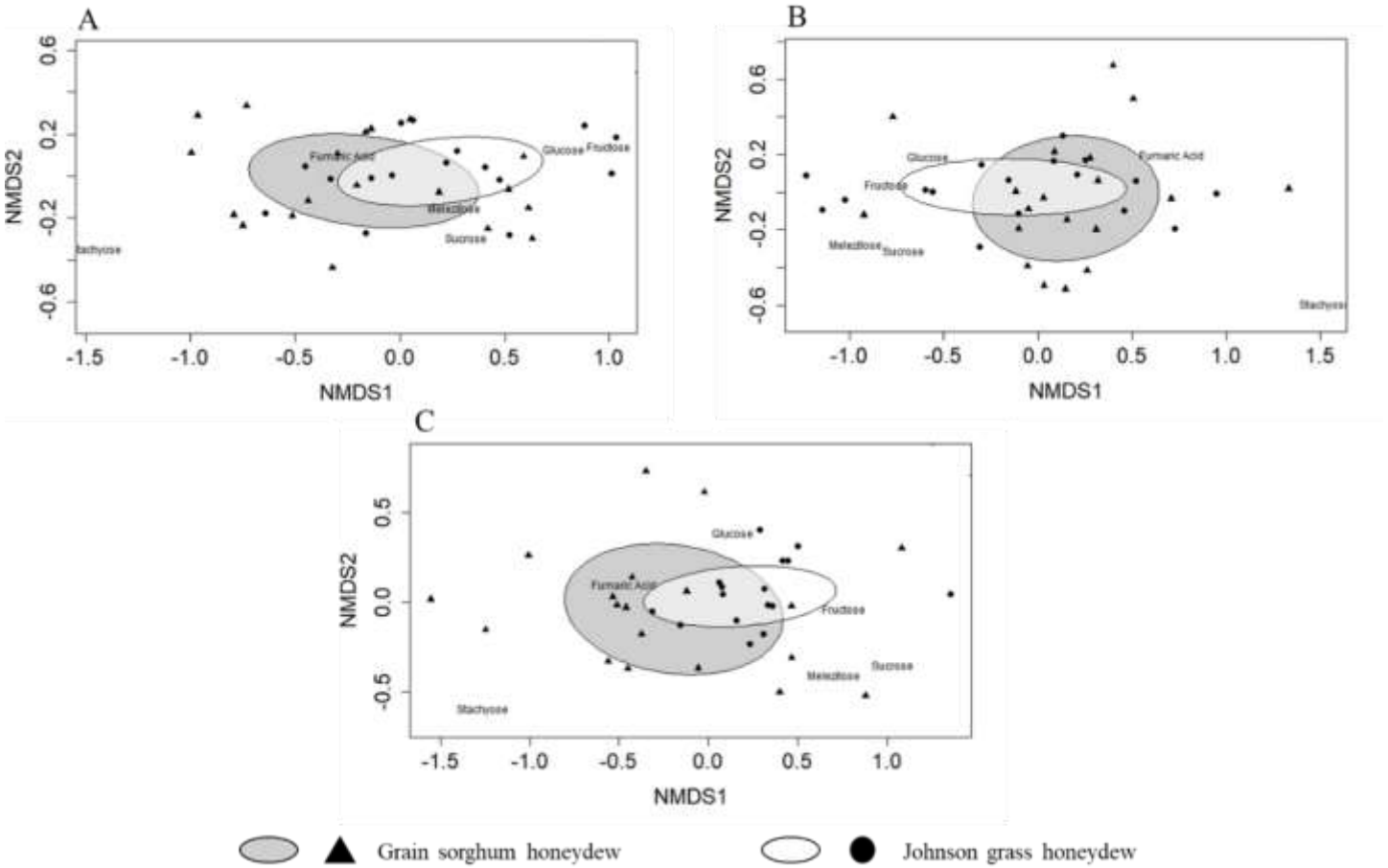


Figure II-2 Non-metric multidimensional scaling (NMDS) ordination diagram. Sugar and organic acid profiles are similar between grain sorghum and Johnson grass honeydew collected after 24 (A) and 72 (B) hours. Profiles are significantly different between grain sorghum and Johnson grass honeydew collected after 120 hours (PERMANOVA F = 3.7925; df = 1,34; P = 0.01) (C).

Table II-2 Sugar and Organic Acid Concentrations (mg/ml) of grain sorghum and Johnson grass SA Honeydew Over Time. Values are mean ± SE of the mean. As per one-way ANOVA, bolded values within timepoints indicate statistical significance at $p < 0.05$.

	24 hours		**72 hours**		**120 hours**	
Sugar/ Organic Acid	Grain sorghum	Johnson grass	Grain sorghum	Johnson grass	Grain sorghum	Johnson grass
Fructose	0.66 ± 0.71	1.18 ± 0.73	2.47 ± 0.71	**4.67 ± 0.68**	3.32 ± 0.73	4.87 ± 0.70
Glucose	0.76 ± 0.66	1.3 ± 0.68	2.39 ± 0.66	4.32 ± 0.63	2.91 ± 0.68	4.55 ± 0.64
Melezitose	0.1 ± 0.10	0.11 ± 0.10	0.39 ± 0.10	0.43 ± 0.10	0.35 ± 0.12	0.52 ± 0.10
Stachyose	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Sucrose	1.36 ± 0.99	1.34 ± 0.96	3.47 ± 0.96	4.58 ± 0.89	3.15 ± 0.96	4.17 ± 0.91
Fumaric Acid	1.16 ± 0.35	**1.82 ± 0.37**	2.39 ± 0.36	3.24 ± 0.34	2.34 ± 0.37	**3.76 ± 0.35**

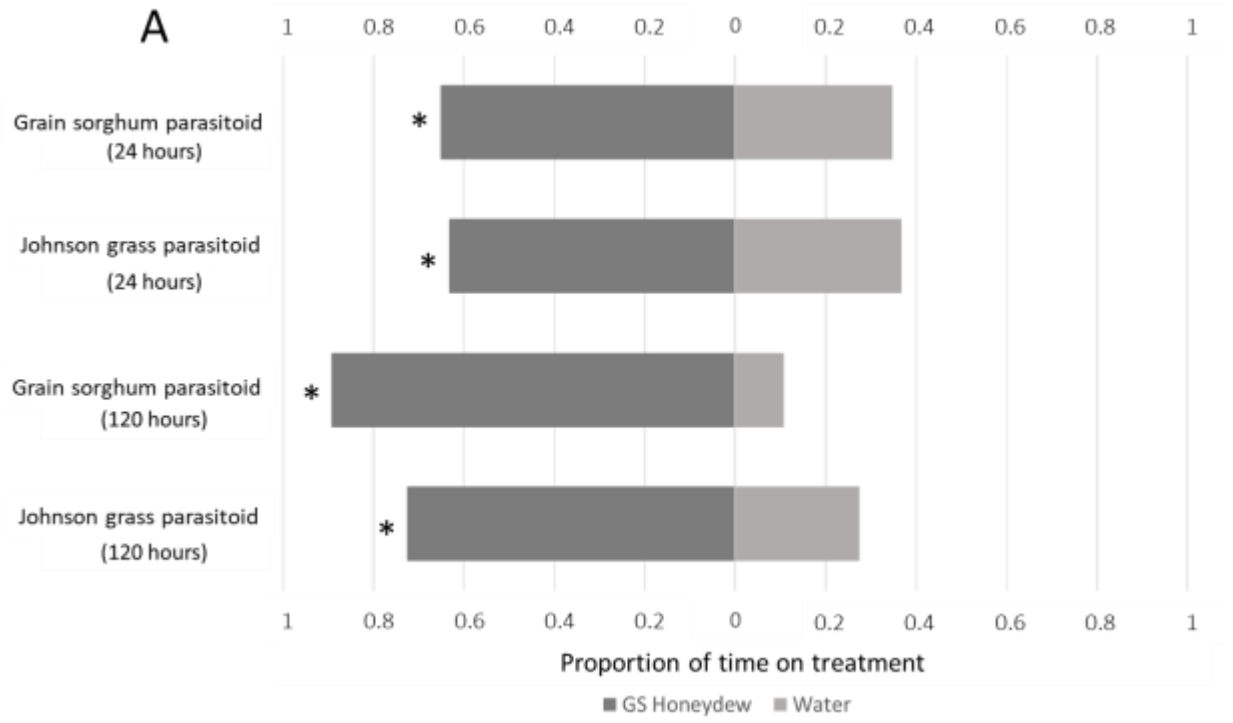
Table II-3 Proportional Composition of Amino Acids in grain sorghum and Johnson grass SA Honeydew Over Time. Values are mean proportions ± SE of the mean. As per one-way ANOVA, bolded values indicate statistical significance at $p < 0.05$.

	24 hours		**72 hours**		**120 hours**	
Amino Acid	Grain sorghum	Johnson grass	Grain sorghum	Johnson grass	Grain sorghum	Johnson grass
Alanine	0.09 ± 0.03	0.03 ± 0.03	0.06 ± 0.03	0.1 ± 0.03	0.01 ± 0.04	0.02 ± 0.03
Arginine	0.02 ± 0.02	0.02 ± 0.02	0.07 ± 0.02	0.05 ± 0.02	0.02 ± 0.02	0.01 ± 0.02
Asparagine	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Aspartic Acid	0.08 ± 0.02	0.14 ± 0.02	0.01 ± 0.02	0.09 ± 0.02	0.13 ± 0.03	0.11 ± 0.02
Glutamic Acid	0.16 ± 0.03	0.16 ± 0.03	0.11 ± 0.03	0.14 ± 0.03	0.16 ± 0.03	0.15 ± 0.03
Glutamine	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.17 ± 0.01
Glycine	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
Histidine	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.11 ± 0.01
Isoleucine	0.01 ± 0.003	-	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.004	0.01 ± 0.003
Leucine	0.01 ± 0.003	0.01 ± 0.002	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.004	0.02 ± 0.004
Lysine	-	0.01 ± 0.002	-	-	0.01 ± 0.003	-
Phenylalanine	0.01 ± 0.01	-	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Proline	0.39 ± 0.07	0.34 ± 0.07	0.31 ± 0.07	0.3 ± 0.07	-	0.02 ± 0.07

Serine	0.07 ± 0.04	0.15 ± 0.04	0.14 ± 0.04	0.12 ± 0.04	0.35 ± 0.04	0.12 ± 0.04
Threonine	0.01 ± 0.001	0.02 ± 0.006	0.02 ± 0.006	0.04 ± 0.006	0.03 ± 0.007	0.01 ± 0.006
Tryptophan	0.01 ± 0.003	-	0.01 ± 0.003	-	0.02 ± 0.003	**0.09 ± 0.003**
Tyrosine	**0.02 ±0.003**	0.003 ± 0.003	0.02 ± 0.004	0.01 ± 0.004	0.04 ± 0.004	0.03 ± 0.004
Valine	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01

Table II-4 Neither timepoint nor original parasitoid aphid host mediate parasitoid preference (measured as the relative proportion of time spent on each treatment) for grain sorghum or Johnson grass honeydew versus water. Bolded values indicate statistical significance at $p < 0.05$.

	ANCOVA		
Variables	F value	df	P value
Collection Timepoint	1.096	1,71	0.2986
Original Parasitoid Aphid host	0.6485	1,71	0.4223
Collection Timepoint x Original Parasitoid Aphid host	0.4947	2,104	0.6112
Choice Treatment	0.0729	1,71	0.7879
Honeydew Concentration (covariate)	4.9370	1,71	**0.0295**



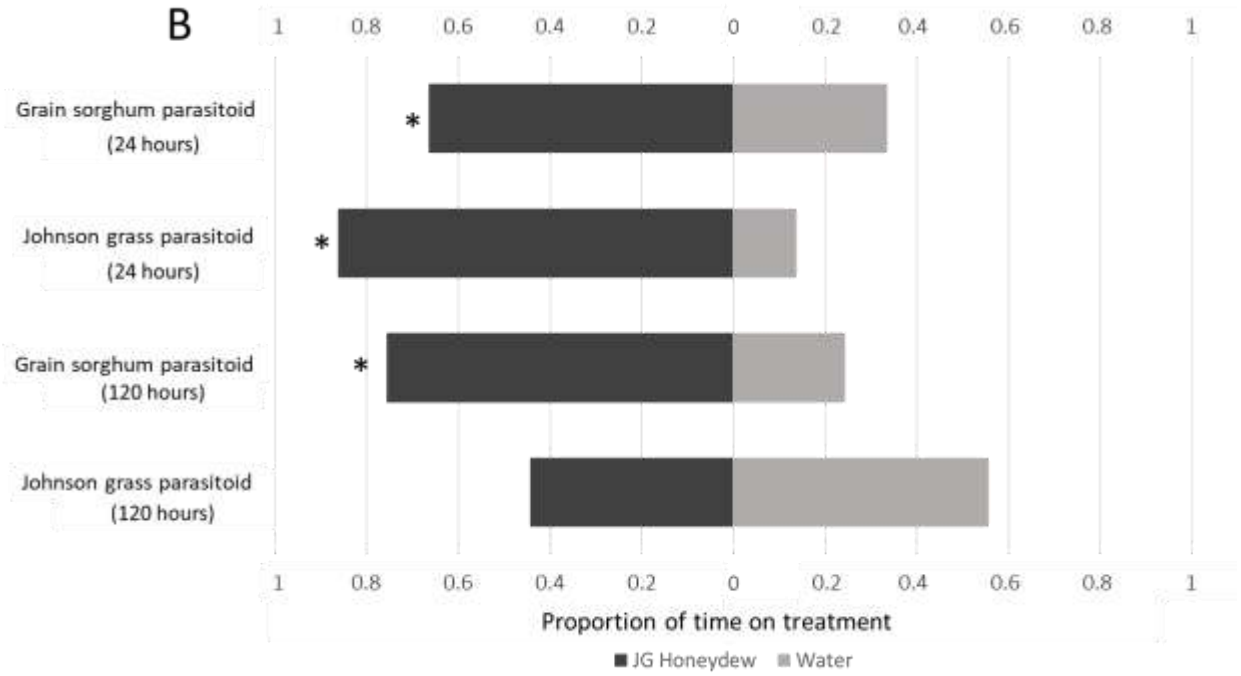


Figure II-3 (A) Grain sorghum and Johnson grass parasitoids are both attracted to grain sorghum honeydew. (B) Grain sorghum and Johnson grass parasitoids are mostly attracted to Johnson grass honeydew. As per a one-sample t-test, an asterisk indicates statistical significance at $p < 0.05$.

Table II-5 Neither timepoint nor original parasitoid aphid host mediate parasitoid preference (measured as the relative proportion of time spent on each treatment) for grain sorghum versus Johnson grass honeydew

	ANCOVA		
Variables	F value	df	P value
Collection Timepoint	1.107	1,35	0.300
Original Parasitoid Aphid host	3.616	1,35	0.066
Collection Timepoint × Original Parasitoid Aphid host	1.331	1,35	0.256
Honeydew Concentration (covariate)	0.136	1,35	0.714

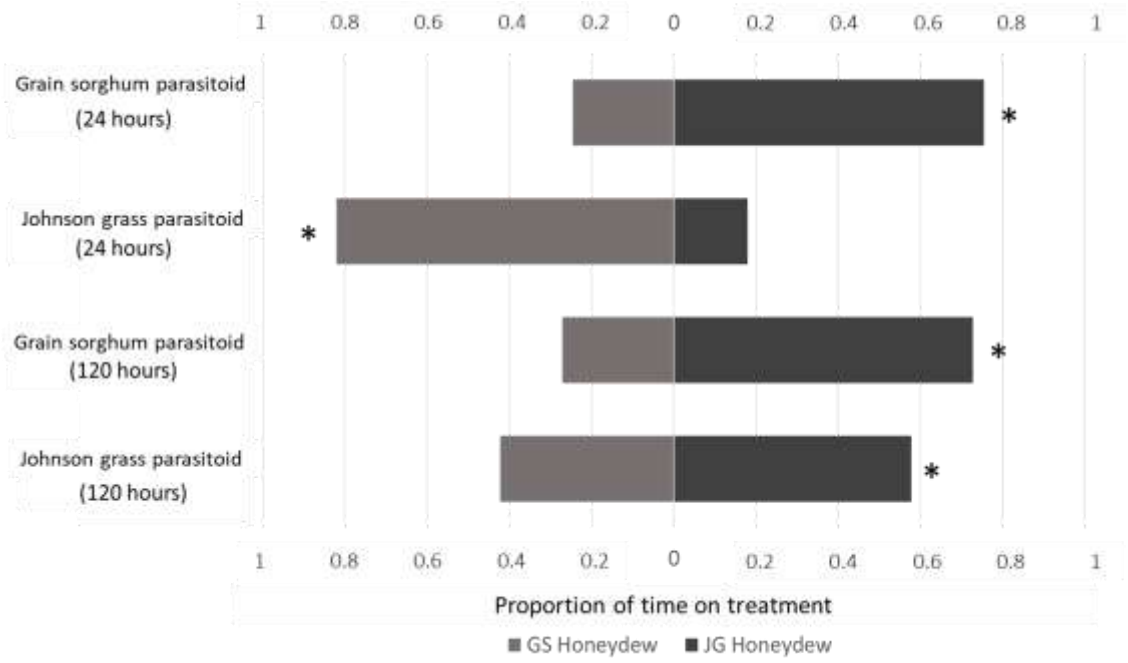


Figure II-4 Both grain sorghum and Johnson grass parasitoids preferred Johnson grass honeydew, with the exception of Johnson grass parasitoids at the 24-hour timepoint (one-sample t-test). As per a one-sample t-test, an asterisk indicates statistical significance at $p < 0.05$.

Discussion

Aphelinus nigritus preferred sorghum aphid honeydew produced by SA feeding on Johnson grass over that of SA feeding on grain sorghum. This preference does not appear to be mediated by differences in honeydew sugar, amino acid, or organic acid composition between honeydews from SA feeding on Johnson grass or grain sorghum. The sugar and organic acid profiles were mostly similar between honeydews produced by SA feeding on sorghum and Johnson grass, except for honeydews collected after 120 hours. Differences at this timepoint appear to stem from a greater accumulation of fumaric acid in Johnson grass honeydew. No study to my knowledge has assessed the role of fumaric acid as a parasitoid attractant or repellant. Consequently, it is unknown as to whether this organic acid affects parasitoid recruitment to

Johnson grass honeydew. Amino acid profiles were similar between host plants across all timepoints. About 70% of all detected amino acids consisted of aspartic acid, glutamic acid, proline, and serine —all nonessential amino acids for aphids [60, 99, 127], which obtain essential amino acids from the obligate bacterial endosymbiont *Buchnera aphidicola* [128]. Further, aspartic acid, glutamic acid, and proline are likely not limiting amino acids, having all been detected in high amounts on several cultivars of grain sorghum [129, 130] and detected in Johnson grass honeydew samples in this study.

With respect to honeydew sugar concentration, I observed a higher concentration of total honeydew sugars in Johnson grass. Higher honeydew sugar concentrations may result from generally higher concentrations of sugar in Johnson grass leaves. However, despite studies separately assessing sugar concentration in Johnson grass rhizomes and leaves [131, 132] and grain sorghum kernels [133], direct measurements of differences in phloem sugar concentration between these two plant species have not been attempted, to my knowledge.

Through the honeydew preference bioassays, it was evident that honeydew from either plant source is attractive to *A. nigritus*. In the honeydew versus water choice tests, honeydew from aphids fed on the two host plants tested elicited *A. nigritus* feeding and searching behaviors. The specific sugar requirements of this parasitoid have not been reported. However, several aphelinids readily consume and display searching behaviors on host honeydew [134-136], suggesting that *A. nigritus* may use honeydew as a cue to locate SA. This is not always common in parasitoids, likely due to high levels of oligosaccharide sugars that may reduce honeydew

palatability [137]. Along with crystallization, high viscosity can also make honeydew sugars difficult to consume [102], which is why parasitoids tend to prefer nectar resources [138]. However, during SA infestations, SA honeydew is likely the most reliable and widely available sugar resource for *A. nigritus* inhabiting grain sorghum and Johnson grass fields. Even if nectar or extra floral nectar is available along field margins, *A. nigritus* may choose the sugar option closest to their host and present in high abundances, as seen in other parasitoids [106]. This is likely because for synovigenic parasitoids (i.e., females are not born with their full complement of eggs), reducing energy use and host searching costs by remaining within host patches may increase the number of eggs they oviposit, creating selective pressure to evolve honeydew consumption instead of reliance on nectar and the associated active searching for flowers [139, 140].

The original parasitoid aphid host does not generally affect this parasitoid's preference for an available sugar resource. While sugar requirements specific to *A. nigritus* have not been reported, species of Aphelinidae are known to consume glucose, fructose, and sucrose [136, 141, 142]. It is important to remember that *A. nigritus* is synovigenic, and would require some source of sugars to mature eggs [143]. Thus, females would likely not reject available honeydew, irrespective of the host plant. This idea is supported by comparisons of the honeydew versus water experiments, where parasitoids displayed similarly strong preferences for either grain sorghum or Johnson grass honeydew over water.

When given a choice between grain sorghum and Johnson grass honeydew, most parasitoids preferred Johnson grass honeydew. This is surprising as it directly contrasts field observations of higher *A. nigritus* parasitism rates on grain sorghum (B. Elkins, unpublished data). Differing field parasitism rates may reflect physical instead of chemical plant traits. Compared to grain sorghum, mature Johnson grass has more bicellular trichomes that can lead to microroughness on the leaves [144] Leaf surface can influence parasitism rates of the same host feeding on multiple plants. For example, Mulatu et al. [145] observed lower instances of parasitism in the potato tuber moth, *Phthorimaea operculella* when feeding on tomato versus potato leaves. This is attributed to a high density of glandular trichomes on tomato that deter parasitoid visitation. Differences in leaf surface may also significantly slow parasitoid movement, which could make finding hosts difficult [146]. Although parasitoids generally prefer plants on which they produce more offspring [147-149], this is not always the case [150]. In this system, it appears that *A. nigritus*'s honeydew preferences do not align with their host oviposition preference.

The only exception to *A. nigritus*'s preference for Johnson grass honeydew were parasitoids originating from Johnson grass, which preferred grain sorghum over Johnson grass honeydew collected after 24hours. This result, along with the observations of no preference in Johnson grass parasitoids between Johnson grass honeydew collected after 120 hours and water are perplexing. Since honeydew is a suitable medium for microbial growth [101, 156, 157], these results were initially presumed to stem from differences in the microbial compositions of grain sorghum and Johnson grass honeydews. Available data on the microbial compositions of both honeydews at different timepoints (J. Holt, unpublished data) does not evidence a correlation

with parasitoid preferences measured in this study. As opposed to microbes themselves, my results concerning parasitoid preferences may stem from the presence of microbial metabolites or microbial volatile organic compounds (MVOCs), which are both shown to mediate natural enemy activity. For example, some metabolites of the bacterium *Bacillus thuringiensis* repel and deter oviposition of *Eretmocerus eremicus* parasitoids on whiteflies [158]. With respect to volatiles, Fand et al. [159] isolated VOC-producing bacteria from grapevine mealybug honeydew that was highly attractive to the endoparasitoid *Anagyrus dactylopii*. Similarly, Leroy et al. [101] isolated microorganisms from pea aphid honeydew that attracted and enhanced predation by hoverflies. Based on this chapter's results, the microbial metabolites or MVOCs present in SA honeydew should be assessed and their roles in mediating *A. nigritus* honeydew preferences tested.

Despite an apparent host preference for SAs feeding on grain sorghum, *Aphelinus nigritus* attraction to Johnson grass SA honeydew has positive implications for pest management, as it suggests that this honeydew can recruit parasitoids to SA-infested plants. Ideally, this could lead to augmentation of parasitoid populations to potentially suppress SA on Johnson grass before it spills over to grain sorghum during the crop's growing season. Whether *A. nigritus* honeydew attraction can lead to SA suppression is another relevant question that should inform any assessments of this parasitoid's potential as a biocontrol agent of SA.

CHAPTER III

APHELINUS NIGRITUS INDUCES TRANSGENERATIONAL FECUNDITY COMPENSATION IN PARASITIZED MELANAPHIS SORGHI

When threatened, aphids adopt a series of behavioral, physiological, developmental, and/or morphological responses against their natural enemies [160, 161]. Depending on the degree of enemy threat, prey may engage in first-line, second-line, or last resort responses. First-line responses are immediate and typically include behavioral responses such as dropping off plants [68], rhythmic kicking [162], or abdomen bucking [163], or chemical responses such as the production of cornicle secretions. In some aphids, cornicle secretions bear alarm pheromones that alert conspecifics of impending danger [164]. Cornicle secretions can also coat the face and mouthparts of certain predators and prevent predators' molting or lead to starvation [64]. While first-line responses can be successful, they are often bypassed by some aphid parasitoids. For example, the kicking and bucking defensive behaviors of *Diuraphis noxia*, do not reduce rates of parasitism by *Aphelinus asychis* and *Aphidius matricariae* [165]. Some parasitoids are also attracted to the (E)-β-farnesene (EBF) component of the aphid alarm pheromone [166] facilitating parasitism [167]. In situations where first-line responses are overcome, aphids may proceed with second-line immunological responses.

Second-Line Immunological Response

In many insect hosts, immunological responses include the initiation of encapsulation and melanization responses [62, 168]. During encapsulation, host hemocytes surround a developing parasitoid. Melanin is then deposited around the parasitoid egg or larva to asphyxiate the intruder

[62, 169]. Launching immunological responses is costly [170, 171], as they may negatively affect insect host size, fecundity, and mating success [172]. In cases of super parasitism (i.e., conspecific females ovipositing in a previously parasitized host), the insect host immune system may not effectively respond to parasitoid threats [173, 174], especially when other factors like pesticide exposure or predation threat are at play [175]. Similarly, the dual presence of predators and parasitoids can overwhelm host responses and ultimately suppress immune function [176, 177]. Aphids lack strong encapsulation responses against parasitism [178, 179]. However, it is worth noting that some aphids compensate for immune deficiencies by forming mutualisms with bacterial endosymbionts. Nonetheless, the effectiveness of symbiont-mediated responses varies by parasitoid type. As an example, pea aphids, *Acyrthosiphon pisum* containing the bacterial endosymbiont, *Hamiltonella defensa*, can survive parasitism by *Aphelinus ervi*. However, *H. defensa* provides no protection against *Praon pequodorum* [180]. A similar case is seen with *Aphis fabae*; when hosting *H. defensa*, the aphid is protected from *Aphidius colemani*, but not from *Lysiphlebus fabarum* attacks [181].

Third-Line Fecundity Response

When behavioral, chemical and immunological responses fail, aphids and other insect hosts may trade survival for increased reproduction [182]. Parasitism effects are often not immediate, but rather increase over the time of host infection [183]. Therefore, hosts have a short window of opportunity to utilize resources towards increased fecundity. For example, parasitized *Drosophila nigrospiracula* males court more females and invest more reproductive effort than unparasitized controls [184]. Similarly, following high-dose infections of the pathogen *Serratia marcescens*, *Gryllus texensis* crickets tend to lay more eggs [185]. Among crustaceans the water

flea, *Daphnia magna,* exhibits fecundity compensation when parasitized by the horizontally transmitted microsporidian, *Glugoides intestinalis* [186]. In certain cases, parasitism is not necessary for compensation; a simple mechanical injury like small needle punctures can induce reproduction. For example, pea aphids wounded with needles accelerate reproduction of live offspring [70]. When punctured with a needle containing gram negative (G-) bacterium *Enterobacter cloacae*, punctured aphids continually increase their reproductive rate relative to uninfected controls [187]. In some aphids, fecundity compensation may be delayed till the next generation. For instance, soybean aphids, *Aphis glycines*, parasitized by *Lysiphlebus orientalis* exhibit transgenerational fecundity compensation, wherein their surviving offspring exhibit higher fecundity than offspring from non-parasitized aphids [188]. Similarly, the offspring of cowpea aphids, *Aphis craccivora*, reproduce more when parents are parasitized by *L. orientalis or Lysiphlebus fabarum* [189]. Whether direct or transgenerational, this physiological response often comes with a cost, as infected aphids typically have shorter longevity than controls [187]. However, in some aphid species, fitness (measured as reproductive success) is ultimately higher among parasitized individuals.

Potential for Fecundity Compensation in SA

SA is an anholocylic species, wherein nymphs are born alive (viviparous), and females reproduce parthenogenetically [40]. In the presence of *A. nigritus* parasitoids, SAs display a series of bucking and kicking behaviors (pers. observation). However, *A. nigritus* usually bypasses these first line responses and oviposits in its aphid hosts. SA encapsulation responses have not been studied, and the aphid does not possess any of the known facultative endosymbionts like *H. defensa*, which may protect against the parasitoid [190]. As seen in other

aphid studies [70, 188], SAs may respond to natural enemy attack by increasing their fecundity. In this study, I assessed whether fecundity compensation occurs in SA populations. I tested for fecundity compensation by first simulating aphid wounding with a needle puncture, then exposing aphids to parasitism by *A. nigritus*. Based on prior studies, I hypothesized that aphids punctured by a needle and stung by *A. nigritus* would produce more offspring than corresponding unpunctured/unstung controls. Additionally, I hypothesized an increase in aphid reproduction among the F_1 generation of needle punctured/*A. nigritus*-stung aphids.

Materials and Methods

SAs occurring on grain sorghum, *Sorghum bicolor*, at the Texas A&M University Farm (TAMU Farm) in Somerville, Texas (30°32'58.0"N 96°26'01.6"W) were collected in fall 2018 and reared on DEKALB® DKS 4420 (Bayer, St. Louis, MO) grain sorghum plants in a 34.3 x 34.3 x 61 cm rearing cage (BioQuip, Rancho Dominguez, CA) to establish a main colony from which clonal colonies were derived. The cage was placed on a shelf in a rearing room at 27°C under a 16:8 light-dark cycle.

Establishment of Clonal Colonies

To take into account the potential effects of aphid genotype on the results [191], pairs of 5-day old SAs born to the same mother (i.e. sister clones) were used for control and treatment replicates. To generate sister clones and standardize SA age, adult apterous aphids were transferred from the main colony to separate three-week-old sorghum plants in a 3.8 x 20.96 cm-high cone-shaped cell (Amazon, Seattle, Washington), and enclosed in a 1-liter plastic bottle. The base of the bottle was removed and covered with mesh fabric (Joann, Hudson, Ohio) and

placed on a separate shelf in a rearing room under the same conditions mentioned above. Each aphid was monitored daily for survival and reproduction. Aphids who died before producing nymphs were replaced. 5-day old aphid sister clones were removed in pairs from each plant and used for needle puncture or parasitism experiments. In cases in which aphid mothers produced more than 1 pair of sister clones, each pair was considered a separate experimental replicate (i.e., an aphid producing 8 nymphs would generate 4 pairs of sister clones for use in 4 separate replicates). Pairs of sister clones from approximately 30 different aphid mothers were used as replicates across needle puncture and parasitism experiments.

Establishment of Aphelinus nigritus colonies

Approximately 30 mummies containing *A. nigritus* were collected from aphid-infested grain sorghum plants growing in greenhouses outside the Texas A&M Entomology Research Lab (30°36'52.5"N 96°21'02.9"W). All mummies were placed in a Petri dish and stored in a plastic bag with a damp paper towel to prevent desiccation. After emerging from a mummy, each parasitoid was identified with a dichotomous key and added to a 34.3 x 34.3 x 61 cm rearing cage (BioQuip, Rancho Dominguez, CA). Species IDs were verified by Dr. Jim Wooley, (Texas A&M University). *A. nigritus* was reared on SA-grain sorghum infested plants (from the aphid colony described above) in a growth chamber at 27°C under a 16:8 light-dark cycle. Rearing cages were supplemented with aphid-infested grain sorghum plants as needed.

SA Needle Wound Experiment

F_0 parental generation: One aphid from each pair of 5-day old sister clones was placed on an experimental arena where they were left undisturbed for five minutes to acclimate [188].

Observation arenas consisted of a 6 mm diameter hole drilled on the left side of a Petri dish. Two-week-old sorghum plants, grown in 50ml centrifuge tubes (Thermo Fisher, Waltham, MA) with bottoms cut off for drainage, were passed through the Petri dish hole and secured with adhesive putty (Figure III-1). The combined plant tube-Petri dish set up was taped to a tray for easy handling and transport. Under a dissecting microscope, each aphid was wounded once dorso-laterally with a 0.15 mm diameter needle (Bioquip, Rancho Dominquez) attached to a bamboo skewer. The wounding site was approximately 1 mm deep and always on the right side, just above the cornicle [188]. The needle was cleaned with 95% ethanol, then dipped in cold autoclaved water before each wounding. The Petri dish was then covered and placed inside a growth chamber under the previously stated conditions. Control replicates were placed in experimental arenas but not wounded by needles. Nymphal production was only recorded for 1 week to assess immediate responses to mechanical wounding. Nymphs produced each day between needle wounding and control treatments were removed with a paintbrush.

F_1 generation: One neonate nymph from each wounded aphid was placed in a separate experimental arena containing a sorghum leaf. These neonate nymphs represented the F_1 generation, so were allowed to develop to the adult stage, without being subjected to wounding [188]. Fecundity was recorded daily for 1 week to assess transgenerational fecundity compensation, if it occurred. As with the parental generation, all nymphs produced each day by treatments and controls were removed with a paintbrush and counted.

SA Parasitism Experiment

F_0 parental generation: As in the needle wounding experiment, an aphid from each pair of 5-day old sister clones was placed on individual experimental arenas containing sorghum leaves. Aphids were allowed to settle for 5 minutes, after which one *A. nigritus* wasp (cooled at -20 °C for 20 seconds before handling) was placed into the experimental arena. The female was observed under a dissecting microscope until her ovipositor pierced the aphid. *Aphelinus nigritus* can sting to either host-feed or oviposit. If feeding on host hemolymph, females insert the ovipositor for about five minutes, which usually leads to quick host death. In contrast, an oviposition sting lasts about 1 minute [192]. To control for host feeding, only aphids stung for less than 1 minute were selected as replicates. Since *A. nigritus* oviposition stings do not always lead to parasitism (Hopper, pers communication), aphids that were stung and later mummified (successfully parasitized) (17 replicates in this study) were separated from aphids that were stung but did not mummify (16 replicates). As such, fecundity compensation was measured under two conditions: 1) aphids that mummified, and 2) aphids that did not mummify. Post-sting, wasps were removed with an aspirator, and experimental arenas were covered to prevent aphid escape. Control replicates were placed in experimental arenas but not stung by *A. nigritus*. Nymphal production of eventually-mummified aphids and control aphids was recorded for 4 days, after which mummified aphids stopped producing offspring. As in the needle wounding experiment, nymph production between stung but not mummified aphids and controls was recorded for a week. Nymphs were removed each day in both experiments.

F_1 generation: One nymph from each mummified or stung but not mummified aphid was placed in a Petri dish. These nymphs represented the F_1 generation. Nymphs were not stung by *A.*

nigritus and allowed to develop to the adult stage. Fecundity was recorded between treatment and controls for 1 week. All nymphs produced by F_1 aphid mothers were removed each day with a paintbrush and counted.

Using the JMP®, Version 15.2 statistical software (SAS Institute Inc., Cary, USA), separate ANOVAs measured differences in nymphal production between needle wounding and parasitism treatments in the parental and F_1 generations For each generation, the response variable was the difference in nymphal production between treatments and controls. The explanatory variable was wound type (i.e., needle wounding or parasitoid sting). Mummification was nested with wound type to account for aphids who were mummified after a parasitoid sting. Post-hoc, the average number of produced nymphs from needle wounding and parasitoid experiments was compared against controls (between each pair of sister clones) under a one-sample t-test with Bonferroni corrections (null hypothesis = no difference in average number of nymphs produced between treatment and controls). For the parasitism treatment, separate t-tests assessed differences in reproduction between treatments and controls for mummified and stung but not mummified aphids. A one-sample t-test also compared nymphal production by the F_1 generation against controls.

Figure III-1 Experimental setup for needle puncture and parasitoid experiments.

Results

Fecundity Compensation Occurs in F_1 Generations, but Only after Parasitism

In the parental generation, rates of nymphal production between treatments and controls did not significantly differ by wounding type (F = 1.461; df = 1,62; P = 0.2316). Within treatments, neither needle wounding nor *A. nigritus* sting (regardless of whether aphids were mummified) increased SA fecundity. Aphid mothers wounded with needles and unwounded controls did not significantly differ in fecundity (T = 0.4456; df = 29; P = 0.6593) (Figure III-2 A). Similarly, fecundity of aphid mothers mummified by *A. nigritus* and controls did not significantly differ (T = -0.9983; df = 16; P = 0.3351) (Figure III-2 B). Fecundity of aphid mothers who were stung but not mummified by *A. nigritus* and controls was also not significantly different (T = -0.5831; df = 15; P = 0.5671) (Figure III-2 C). In the F_1 generation, rates of nymphal production between treatments and controls did not significantly differ by wounding type (F = 0.1296; df = 1,62; P = 0.7201). Fecundity of F_1 aphids wounded by needles and controls were similar (T = -1.6828; df = 29; P = 0.1035) (Figure III-3 A). However, fecundity of F_1 aphids whose mothers were

mummified by *A. nigritus* was greater relative to controls (T = 2.5005; df = 16; P = 0.0118) (Figure III-3 B). In contrast, fecundity of F_1 aphids whose mothers were stung but not mummified was not significantly different from controls (T = 0.8612; df = 15; P = 0.4027) (Figure III-3 C).

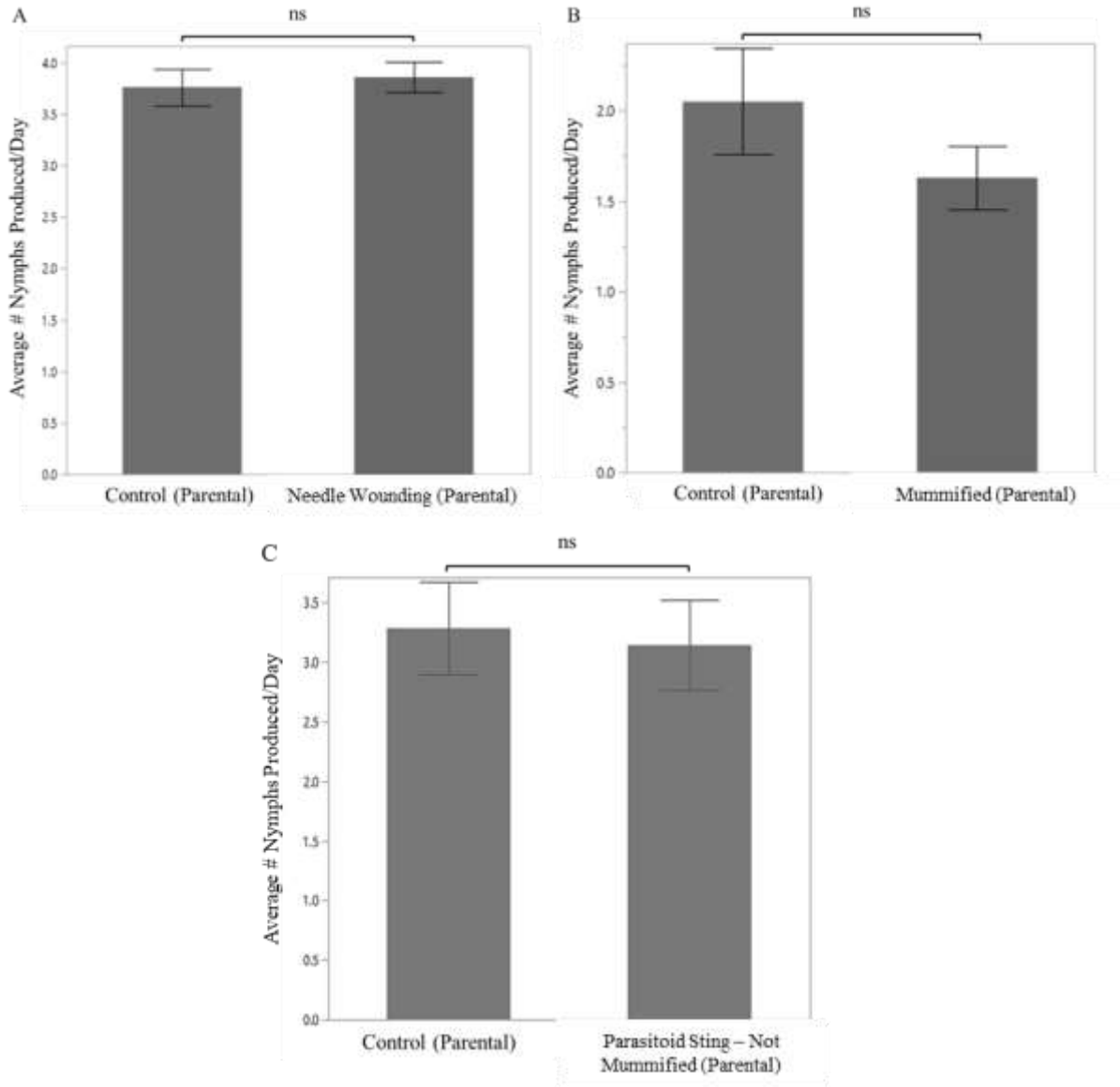


Figure III-2 Average nymphal production per day after needle wounding (A), mummification (B), or a parasitoid sting not resulting in mummification (C) is not significantly different from controls in the parental generation (one-sample t-tests. Statistical significance at $p < 0.05$).

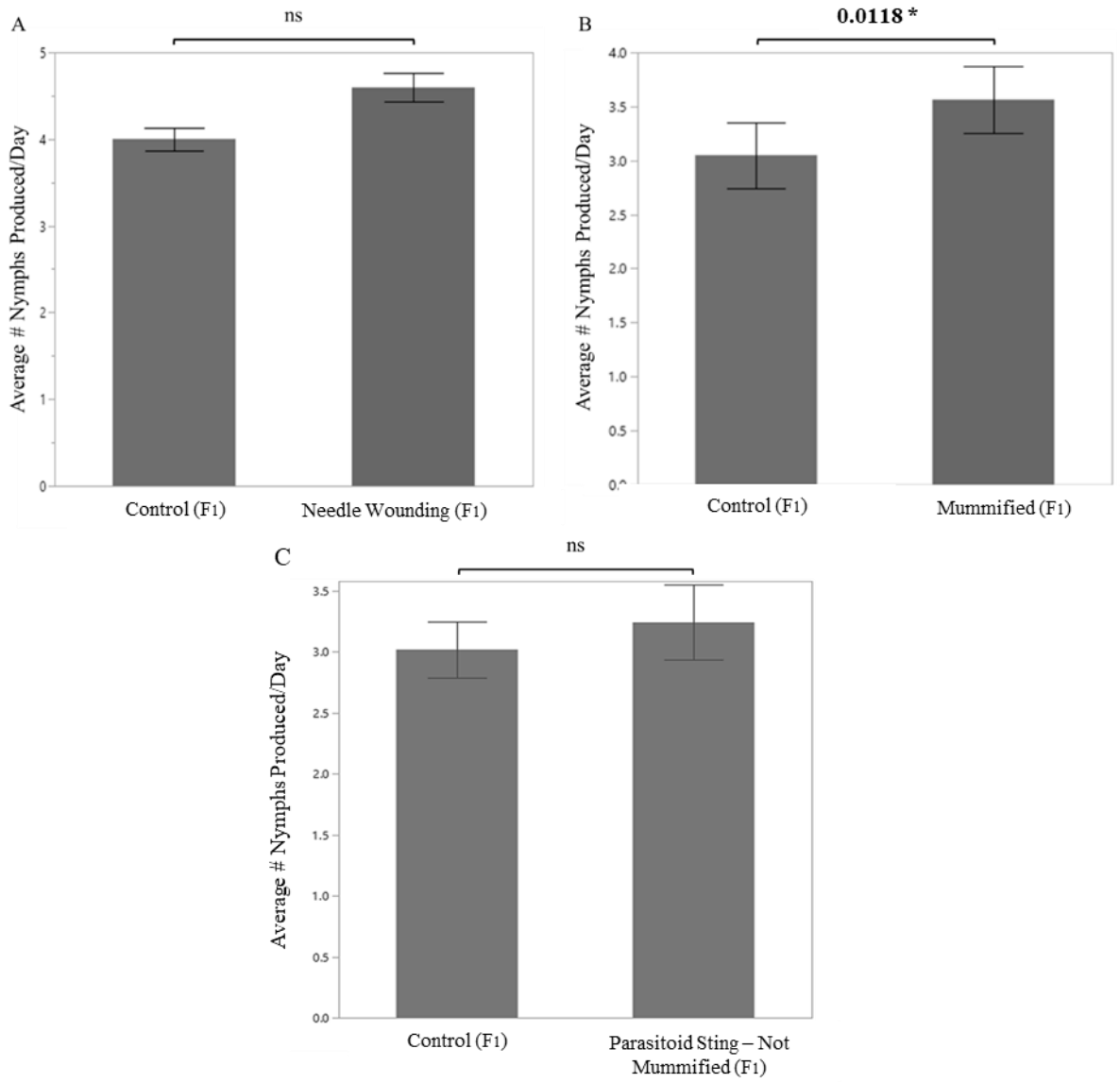


Figure III-3 Average nymphal production per day after needle wounding (A), mummification (B), or a parasitoid sting not resulting in mummification (C) is only significantly different between mummified and control aphids in the F_1 generation (one-sample t-tests. Statistical significance at $p < 0.05$).

Discussion

Transgenerational fecundity compensation was observed in SA when parasitized successfully (i.e. mummification occurred) by *A. nigritus*. In contrast, needle-wounded or stung, but not mummified, SAs did not exhibit increased fecundity relative to controls. Parthenogenetic aphids

like SA have telescoping generations, meaning mothers contain embryos of their daughters and future granddaughters. Thus, the resulting progeny can be subject to adaptive maternal and grandmaternal effects, or transgenerational phenotypic plasticity if primed by environmental stressors to the mother [193, 194]. Priming occurs when the parental generation provides cues that affects the fitness of developing offspring. This has been most commonly reported when the parental environment is subject to biotic stressors [195]. As an example, aphids subject to overcrowding or high predator stress can be primed to increase the production of winged forms [196, 197].

Though much of the literature discusses priming involving the effect of biotic stressors on offspring immune responses [198-200], more attention should be given to reproductive priming, especially with aphids, which generally possess weak immune responses and telescoping generations. Parental priming for enhanced reproduction, leading to fecundity compensation, might improve fitness by increasing the output of nymphs [171], which can theoretically lead to increases in aphid population growth. In this system, the F_1 generation of successfully parasitized SA was primed to increase reproductive output. Moreover, average F_1 fecundity outweighed that of parental aphids, a concerning result that questions *A. nigritus*'s actual effectiveness as a SA biocontrol agent.

No significant difference in fecundity was observed between needle wounded, A. *nigritus* mummified, and A. *nigritus* stung but not mummified aphids in the parental generation. There was also no decrease in SA reproduction in any of our treatments. Altogether, this suggests some level of aphid tolerance to multiple types of wounding. In host-parasite systems, tolerance is

measured as a host's ability to survive and limit the health impact of a parasite [201]. Since aphids are known to possess weak immunological responses when compared to other insects [179], tolerance, as opposed to mounting a costly immune response, in the presence of non-lethal wounding may be an effective host strategy. In this study, SA likely displays tolerance by maintaining nymph reproduction after a wounding event [202, 203]. In terms of nymph size, there was no observable difference in the dry weights of nymphs produced by parental SA between either needle or parasitoid treatments and controls. In the needle treatments, nymphs of needle wounded aphids weighed an average of 20 ± 2.3 µg/nymph compared to nymphs of control aphids which weighed an average of 18.5 ± 2.2 µg/nymph. Nymphs of mummified aphids weighed an average of 16 ± 0.39 µg/nymph compared to nymphs of control aphids which weighed an average of 19 ± 0.66 µg/nymph. Finally, nymphs of stung, but not mummified aphids weighed an average of 17 ± 0.98 µg/nymph compared to nymphs of control aphids which weighed an average of 19 ± 0.75 µg/nymph. Interestingly, there was no difference in average dry weight between nymphs produced by the F_1 generation of mummified SA (16 ± 0.92 µg/nymph) and controls (17 ± 0.83 µg/nymph), suggesting that transgenerational fecundity compensation, while significantly increasing nymphal production, does not decrease nymphal size. Collectively these results suggest that SA, at the very least, can maintain reproduction in the presence of non-lethal and lethal stressors, up until death.

In this study, aphids that were stung but not mummified survived longer than mummified aphids who died approximately 4 days after exposure to *A. nigritus*. However, the aphids that survived *A. nigritus* stings did not produce nymphs at a higher rate than controls or mummified aphids. Similarly, the F_1 generation of aphids whose mothers were stung but not mummified did not

reproduce at higher rates than controls. Considering the lack of effect also observed in aphids (and their offspring) wounded by needles, parasitoid stings, though necessary to trigger transgenerational fecundity compensation, are not by themselves sufficient. Only stings that resulted in parasitism triggered fecundity compensation in SA F_1. This result possibly reflects a differential SA reproductive response to increasing risks of death. Unlike with needle or parasitoid stings alone, the insertion and development of parasitoid larvae probably cued a risk for early death and rapidly shifted resource allocation strategies in successfully parasitized SA [184]. Rapid reproduction in response to increased risk has also been explored by Barribeau et al. [204], who observed fecundity compensation in pea aphids exposed to the predator-induced aphid alarm pheromone (E)-β-farnesene (EBF), but not in aphids wounded by a small gauge sterile needle. These results support the assumption that aphids' perception of risk affects their fecundity.

This study did not assess the presence of *A. nigritus* eggs laid inside aphids that were stung but not mummified. Though it can be inferred that mechanical insertion of the *A. nigritus* ovipositor is not enough to trigger compensation, the presence of unhatched eggs may have introduced teratocytes (i.e., large extraembryonic parasitoid cells released into a host after a parasitoid egg hatches), that constrained the parental environment and generated a cue towards greater F_1 reproduction. Once released from an injected parasitoid egg, teratocytes actively degenerate host tissues and often lead to host castration [205, 206]. Teratocytes can also manipulate the obligate aphid bacterial endosymbiont *Buchnera aphidicola*, which provides essential amino acids for survival and development [128], by increasing and diverting the production of amino acids to feed developing parasitoid larvae [207]. Alternatively, the results may stem from the presence of

A. nigritus venom in parental aphids, which could have primed the life history shift for reproductive compensation in F_1 SA [193]. The injected venom of parasitoids is a widely studied host regulation factor used to degrade host tissues in favor of parasitoid larval development [208]. Venom proteins in some parasitoids, like the γ-glutamyl transpeptidase isolated from *Aphidius ervi* [209], directly induces apoptosis of host ovaries, which may have signaled a high-risk need for increased reproduction among F_1 SA.

While members of the family Aphelinidae typically inject teratocytes and venom during oviposition [210], literature specific to this aspect of *A. nigritus* reproductive physiology is currently lacking. Furthermore, it is unknown if this parasitoid has teratocytes, or if it can sting aphids and inject venom without ovipositing, or vice versa. This information is collectively relevant to assess whether eggs, teratocytes, or venom separately, or in combination can prime increased SA reproduction. Future studies might consider tracking egg insertion in unmummified hosts through light microscopy [208] or transmission electron microscopy [211], to validate teratocyte production by this parasitoid. If confirmed, teratocytes could be cultured *in vitro* and injected into SA to elicit a potential reproductive response [212]. Alternatively, venom function in SA reproduction could be assessed by dissecting and injecting *A. nigritus* venom into SA [213] Altogether, assessing the function of parasitoid eggs, teratocytes, and venom will further uncover key mechanistic factors potentially leading to fecundity compensation in SA.

As a major pest of grain sorghum, SA's ability to tolerate and compensate for *A. nigritus* stings may hamper this parasitoid's effectiveness as a biological control agent. While this study suggests that F_1 daughters of mummified aphids may overcompensate for parasitism, it is

unknown if compensation spans multiple generations. Moreover, any effects of *A. nigritus* mediated fecundity compensation on field SA population growth remains unknown. However, a recent study by Mercer et al. [214] speculates fecundity compensation as a potential explanation for initial observations of increased SA populations post-exposure to *Aphidius colemani* and *Aphidius ervi* biocontrol agents. Whether *A. nigritus* exposure increases SA populations in the field should be assessed to evaluate whether this parasitoid will exert effective control as a biocontrol agent. Hopefully, this work will inspire research on the role of parasitoids in promoting fecundity compensation in other systems using biocontrol agents against other aphid pests. Aphid damage affects multiple crops, including major commodities like soybean, maize, wheat, and cotton [1]. Consequently, the existence of fecundity compensation as a response to biocontrol agents may have already been exacerbating aphid problems under biocontrol programs. Thus, the roles of tolerance and fecundity compensation in aphid population dynamics should be examined and assessed in current and future pest control practices.

CHAPTER IV

TENDING BY RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE), INCREASES LOW POPULATION DENSITIES OF MELANAPHIS SORGHI, AND ITS NATURAL ENEMIES ON GRAIN SORGHUM, *SORGHUM BICOLOR*

In Texas, grain sorghum is visited by multiple ant species, most predominately the red imported fire ant, *Solenopsis invicta* Buren, an invasive species introduced to the southern United States sometime between 1933 and 1940 [215, 216]. Originally from Argentina, RIFA is an aggressive invader that thrives in agroecosystems, primarily because of the mutualisms it establishes with a variety of hemipterans [82, 83, 217, 218]. These mutualisms have led to the displacement of native ant species [82, 83, 219], and to destruction of grassland habitats [220]. RIFAs have been observed tending SA on sorghum in Texas under field (C. Wright, unpublished data) and greenhouse conditions (J. Holt, unpublished data) in what could comprise an emerging mutualistic interaction. Because mutualisms result in net benefits for each interacting species, it is possible that SA populations tended by RIFA could increase to the detriment of grain sorghum production.

Predictably, a RIFA-SA mutualism would be problematic as the density of both species would likely increase, as it has been reported for RIFA mutualisms with the cotton mealybug, *Phenacoccus solenopsis* on Chinese hibiscus [221], and with aphid pests on tomato plants [82]. Mutualism-driven increases in RIFA density may lead to the production of large mounds, which can interfere with crop harvesting [222, 223]. In addition, RIFAs can interfere with aphid natural enemies, as reported for RIFAs tending cotton aphid, *Aphis gossypii*, attacking and reducing the

density of predatory lacewings and lady beetles [217, 224], so facilitating additional increases in RIFA and aphid population densities. RIFA has become a prolific mutualist due in part to a lack of interspecific competition in the US versus their native Argentina, where they often compete with *Camponotus*, *Crematogaster*, *Azteca*, *Cephalotes*, and *Nylanderia* ants [78]. This lack of competition makes RIFAs more likely to develop a food-for-protection mutualism with SA in Texas sorghum.

The effects of RIFA presence on SA natural enemies is presently unknown. As noted previously, several species of natural enemies have been identified attacking this aphid in the US, including *Lysiphlebus testaceipes* Cresson, first reported in Florida [225], *Xanthogramma aegyptium* Wied, first reported in Louisiana [226], and *A. nigritus*, reported in Texas [56]. The fungal pathogen *Verticillium lecanii* (Zimm.) Viegas is cited as a natural enemy in Florida [227]. Reported SA predators include a variety of coccinellids, lacewings, and hoverflies [56]. RIFA tending of SA may adversely affect these natural enemies and hinder SA suppression, as shown in other ant-aphid mutualisms [228, 229]. Thus, it is relevant to assess any effects of RIFA tending of SA on natural enemy density.

Impacts of ant tending on aphids or other hemipterans and their natural enemies can additionally vary with aphid population density. As seen in Breton and Addicott [75], effects of ant tending are typically positive when initial aphid densities are low and per capita ant-aphid interactions are high. Morales [230] observed a similar effect on low densities of *Publilia concave* treehoppers, which benefited more from *Formica obscuriventris* ant tending than at high density populations. Initial population densities of aphids and other hemipterans can also affect

recruitment and density of natural enemies. This is shown in with *Lasius neoniger*-tended *Aphis fabae*, which were better protected from ladybird predation when aphid populations were smaller [231]. This information is applicable to SA, whose population densities vary by season [232]. As such, the extent to which RIFA affects SA population growth and natural enemy density at different SA densities warrants testing.

As noted above, a RIFA-SA mutualism could exacerbate pest problems caused by both RIFAs and SAs, as seen in other examples of ant-aphid mutualisms [82, 221, 233, 234]. This study sought to document the effects of RIFA on SA population density in the field to assess the existence and strength of any potential correlation between species potentially engaging in an incipient mutualistic interaction. Three hypotheses were tested: 1) SA population growth is greater in the presence of red imported RIFA than in its absence; 2) RIFA presence mediates the density of SA natural enemies, and; 3) any effects of SA density on natural enemy density is modulated by initial aphid densities in the field. Documenting an emerging mutualistic interaction between SAs and red imported RIFAs, will serve as a baseline for future, comparative studies.

Materials and Methods

Establishment of Experimental Plots

This study consisted of two identical experiments conducted in summers 2019 and 2020 in a single grain sorghum field (variety Pioneer 83P56), which was not treated with insecticides, in the Texas A&M Research Farm in Somerville, Texas (N30.5519°, W096.4264°). The 2019 experiment was conducted between 19 June and 25 July, while the 2020 experiment was

conducted between 15 June and 23 July; this timing corresponded to midseason GSIII grain filling sorghum stage. Sorghum aphid populations are also at their highest during this period. Three weeks before initiating each experiment, ant presence was assessed using baited centrifuge tubes containing a 1.5cm piece of hot-dog [235]. The centrifuge tubes (50 ml, VWR International, Radnor) were modified by drilling three holes in their caps to allow ant access [236]. Baited traps were attached to the base of one plant in each of 40 randomly chosen plots and were recovered 48 h later; each plot consisted of 5 contiguous plants within a row, and plots were >6 m apart in every direction. Plots in which the baited vial attracted at least five RIFAs were selected for inclusion in the study, and 40 plots were selected in this manner.

RIFAs were excluded from 20 of the 40 plots by applying a Tanglefoot (Scotts Miracle-Gro, Marysville, OH, USA) barrier at the base of each plant within a plot. The barrier was reapplied as necessary, and plants receiving this treatment were repeatedly monitored for presence of ants; additionally, immediately neighboring sorghum plants and all nearby weeds were removed. Sheets of weed barrier ground cover (Dewitt Company, Sikeston) were placed at the base of all plots. A 3-week period from application of the Tanglefoot barrier and the start of the experiment was allowed to ensure that RIFAs were not present in plots corresponding to the RIFA-exclusion treatment (see below).

Measuring RIFA Effects on SA Density and Natural Enemy Activity

Two treatments were used to measure RIFA effects on SA density and natural enemy activity: (i) RIFA-inclusion, and (ii) RIFA-exclusion. Initial SA density was measured 3 weeks after having established the 40 plots (when the Tanglefoot barrier was first applied) and is referred to as

Week 0. Aphid density was measured as the number of aphids on the 5th leaf (from the bottom) in each of the plants per plot. Final SA density was measured 3 weeks later on the same leaves used to measure initial density and is referred to as Week 3. The fifth leaf was used for measuring final aphid density in the few cases (n = 6) in which the 5th leaf died prior to measuring final aphid density.

Additional observations were made to assess whether RIFA presence mediated SA natural enemy activity. Specifically, parasitoid and predator densities were measured on Weeks 0, 1, 2 and 3. The number of successfully parasitized aphids (mummies) was used as a proxy for parasitoid activity, and the combined numbers of live predator adults, immatures, and eggs were used as a proxy for predator activity; parasitoid mummies were of Aphelinidae and Encyrtidae, while predators included Coccinellidae, Syrphidae, and Chrysopidae. All counts of SAs and natural enemies were made with the aid of a manual counter. The average numbers of SAs, parasitoid mummies, and predators across the five plants of each plot were used for data analyses.

The effects of RIFA presence on growth of SA populations were assessed through non-parametric ANOVA, with treatment (RIFA exclusion, RIFA inclusion), year (2019, 2020), and treatment × year interaction as independent variables, and aphid density growth per leaf (= final aphid density – initial aphid density) as the response variable. Aphid density growth values were transformed to rank values prior to analyses, and planned contrast comparisons were used to compare between initial and final aphid densities within each year, if warranted by a significant

treatment × year interaction. Initial aphid densities (Week 0) were compared between years via a Kruskal-Wallis non-parametric ANOVA.

The effects of RIFA tending on any relationship between SA density and natural enemy activity were assessed via Spearman correlations of SA density and parasitoid and predator activities, separately for each RIFA treatment (RIFA inclusion, RIFA exclusion) and year (2019, 2020); all values were converted to their natural logarithm values. Fisher's *r* to *z* transformations were used to compare effect sizes (Spearman's ρ) between RIFA treatments for parasitoids and predators separately, within years. The strength of the responses of parasitoid and predator activities, each separately, were also compared to increasing aphid density between RIFA inclusion and exclusion plots by comparing their linear regression slopes within each of the two years; all values were converted to their natural logarithm values.

The strengths of responses of parasitoids and predators, were compared separately, to increasing aphid densities between seasons with high (2020) and low (2019) aphid densities (see *Results*). This was done by comparing their linear regression slopes between the high and low aphid density seasons for parasitoids and predators separately; all values were converted to their natural logarithm values.

Results

Evidence for a RIFA-SA Mutualism

Initial aphid density was ca. four-fold greater in 2020 (63.6 ± 11.5 aphids per leaf) compared to 2019 (15.6 ± 4.6 aphids per leaf) (Kruskal-Wallis Statistic = 35.253, F = 62.860; df = 1,78; P <

0.0001). Sorghum aphid population growth (slope) differed between the 2019 and 2020 sorghum growing seasons (Table IV-1). Aphid populations grew by 25.7 (± 13.9) aphids per leaf during 2019 but decreased by 59.0 (± 13.9) aphids per leaf during 2020. Although aphid populations did not differ between RIFA treatments (i.e., exclusion *versus* inclusion), the interaction of RIFA treatments with year was significant (Table IV-1). In 2019, aphid populations grew significantly more under RIFA inclusion (57.1 ± 25.0 aphids per leaf) compared to exclusion (–5.6 ± 8.3 aphids per leaf) ($F = 6.845$; $df = 1,76$; $P < 0.0001$), as expected (Figure IV-1). In contrast, aphid population growth did not differ between RIFA exclusion (–67.5 ± 21.5 aphids per leaf) and inclusion (–50.5 ± 8.1 aphids per leaf) plots in 2020 ($F = 0.077$; $df = 1,76$; $P = 0.782$) (Figure IV-1). Collectively, these results showed that red imported RIFA presence variably mediates SA population growth and suggested that RIFA enhances SA population growth only when initial aphid densities are low.

RIFA Mediation of SA-Natural Enemy Activity

A variety of natural enemies were detected in this study. The main aphid parasitoid mummies found were of *A. nigritus*, consistent with previous reports of this parasitoid on grain sorghum in Texas [44, 237]. Among predators, lacewings, hoverflies, lady beetles, and dusky lady beetle larvae were observed, all of which are voracious aphid feeders [56].

Both parasitoid and predator activities were mediated by RIFA presence, though the effect of RIFA presence differed between the two years of this study. Parasitoid activity and SA density were positively correlated in both years irrespective of treatment (i.e., RIFA exclusion or inclusion) but the correlation was 3.2× stronger in the presence compared to absence of RIFA in

2019 (Figure IV-2 A). The opposite occurred in 2020, when the correlation was stronger (1.8×) in the absence of RIFAs (Figure IV-2 B). In the case of predators, predator activity and SA density were positively correlated in 2019, when correlations were slightly (1.5×) stronger in the presence compared to absence of RIFA (Figure 2c). However, predator activity and aphid density were negatively correlated in 2020 but did not differ in the presence compared to absence of RIFA (Figure 2d).

The response of parasitoid activity to increasing SA density (i.e., as measured by the correlation slope) was 2.5× stronger in the presence compared to absence of RIFA in 2019, but unaffected by RIFA in 2020 (Table IV-2). In comparison, the response of predator activity was unaffected by RIFA presence in both 2019 and 2020. Altogether, these results showed variable effects of RIFA presence on natural enemy activity. While RIFA correlations with parasitoid activity at increasing aphid densities are consistently positive, the strength of these correlations and response of parasitoid activity to the presence or absence of ants varies by season. For predators, RIFA correlations with predator activity can be positive or negative depending on the season; regardless, RIFA presence does not significantly affect predator activity.

RIFA Effect on Natural Enemy Activity at Low Initial (2019) Versus High (2020) Initial SA Densities

In the presence of RIFA, the response of parasitoid activity to increasing SA density was positive, and 5.3× greater when initial aphid density was low (2019) versus when it was high (2020) (Table 3). In contrast, while predator activity in the presence of RIFA responded to increasing SA density in both years, it changed signs between years, being positive when initial aphid density was low, and negative when it was high. Overall, these results suggest that RIFA

more strongly enhances positive SA-parasitoid correlations at low compared to high SA densities, and drastically changes correlations between SA density and predator activity from positive to negative at low compared to high SA densities.

Table IV-1: Both initial SA densities and the effects of RIFA treatment on aphid population growth vary by year.

	ANOVA			Kruskal-Wallis test		
Measurements	F value	Df	P value	H value	df	P value
Year	-	-	-	62.86	1,78	< **0.0001**
RIFA Treatment	2.734	1,76	0.102	-	-	-
Year x RIFA Treatment	4.189	1,76	0.044	-	-	-
SA Population Growth (Slope)	98.123	1,76	< **0.0001**	-	-	-

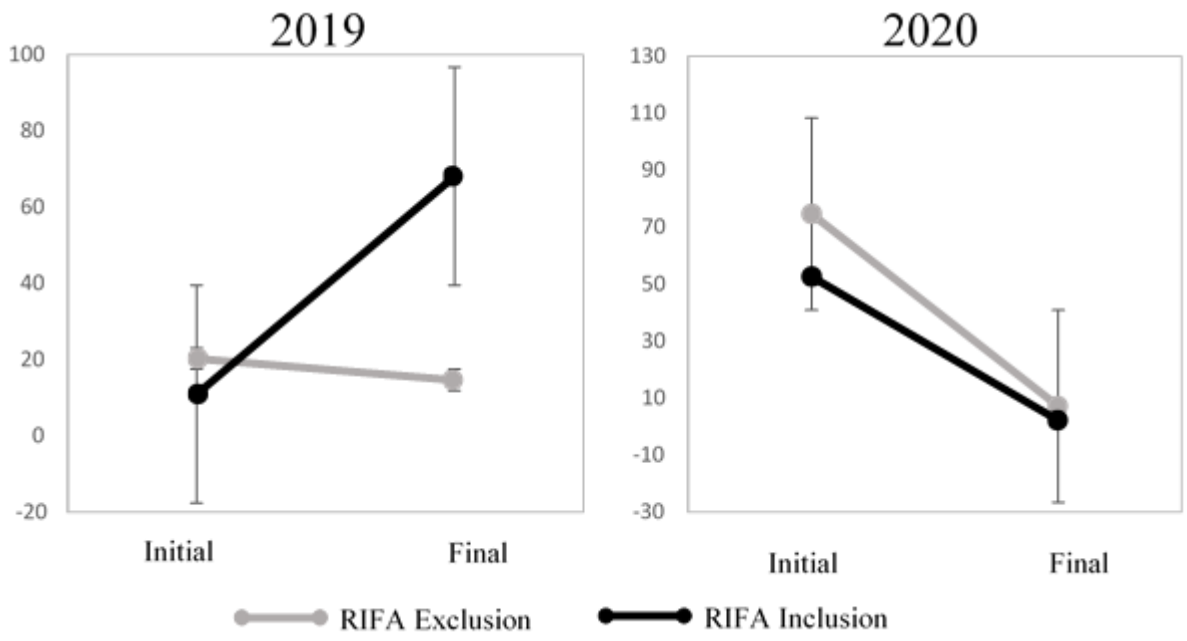


Figure IV-1: A mutualism-led increase in SA populations operates at low SA densities. Final aphid densities are greater when ants are included in 2019 ($F = 4.383$; $df = 1,76$; $P = 0.0396$), but not in 2020 ($F = 1.4107$; $df = 1,76$; $P = 0.2386$). Initial aphid abundances are significantly different between years ($F = 15.0601$; $df = 1,76$; $P = 0.0002$) with 4× less aphids in 2019 than in 2020.

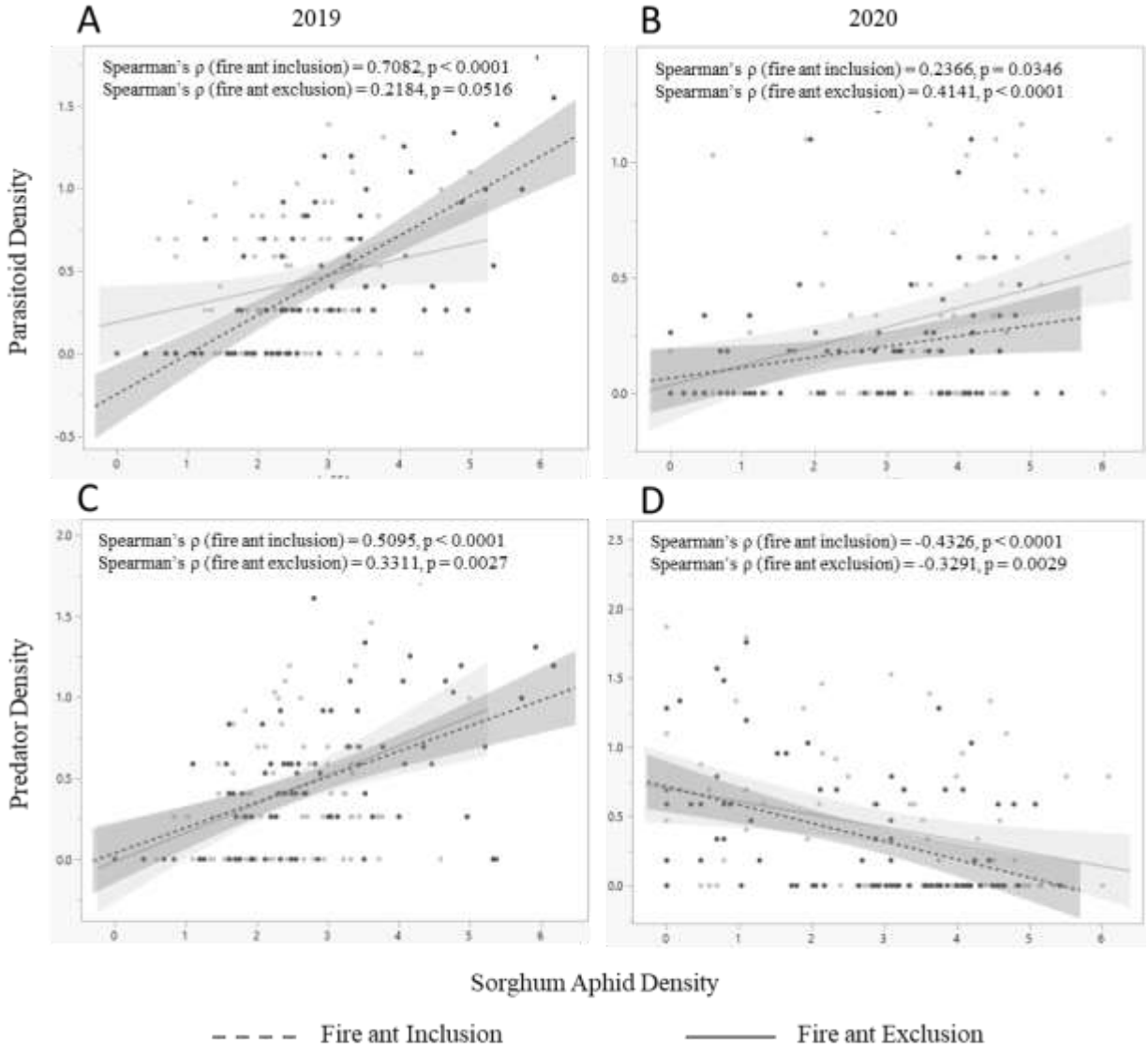


Figure IV-2: Spearman correlations of SA density and parasitoid and predator activities. Parasitoid activity positively correlates with aphid density in 2019 (A) and 2020 (B). Correlation of parasitoid density and SA density is stronger under RIFA inclusion in 2019 (Fisher's $z = 6.22$; $P < 0.001$); Predator activity is variably correlated with aphid density in 2019 (C) and 2020 (D). Correlation of predator density and SA density is stronger under RIFA exclusion in 2019 (Fisher's $z = 2.05$, $P = 0.02$) while correlations were not significantly different in 2020 (Fisher's $z = -1.14$, $P = 0.127$).

Table IV-2: Response of parasitoid activity to increasing aphid density (slope) is stronger in the presence of RIFA in 2019, but not 2020. Response of predator activity to increasing SA density were similar in RIFA inclusion and exclusion in both 2019 and 2020.

				Slope Comparisons		
Year	Natural Enemy	Treatment	Slope	F value	df	P value
2019	Parasitoid	RIFA Inclusion	0.24012	8.18	1,156	**0.005**
		RIFA Exclusion	0.09535			
	Predator	RIFA Inclusion	0.15695	0.15	1,156	0.695
		RIFA Exclusion	0.17865			
2020	Parasitoid	RIFA Inclusion	0.04565	1.45	1,156	0.230
		RIFA Exclusion	0.08340			
	Predator	RIFA Inclusion	-0.13163	0.65	1,156	0.421
		RIFA Exclusion	-0.09344			

Table IV-3: Response of parasitoid activity (slope) is positive at low and high densities but stronger at initially low SA density. The response of predator activity to increasing aphid density is positive at low but negative at high initial aphid density.

			Slope Comparisons		
Natural Enemy	Initial aphid density (Week 0 of experiment)	Slope	F Values	df	P value
Parasitoid	Low	0.240	29.93	1, 156	< **0.0001**

	High	0.046			
Predator	Low	0.157	45.70	1, 156	< **0.0001**
	High	-0.132			

Discussion

Importance of Initial Aphid Density in the SA-RIFA Mutualism

The results showing that SA density increased in the presence of RIFA in the low-SA density year but not in the high-SA density year is consistent with the hypothesis that ant-aphid mutualisms operate only at low aphid densities [75], as well as with suggestions that RIFA only opportunistically tends hemipterans [78, 82, 219]. Differences in the findings between years are likely due to a significantly lower initial abundance of SAs in 2019 versus 2020: Initial SA counts were ca. 15 aphids per leaf in 2019, and ca. 60 per leaf in 2020. A lack of mutualism at higher aphid densities likely stems from a "dilution" of RIFA-SA interactions as compared to lower aphid densities. Aphids are parthenogenetic and have short generation times, so their populations can outnumber those of ants in a relatively short period of time. Per SA-capita RIFA tending levels (and associated effects on SA population growth) may decline simply due to an excess of aphids [238]. In addition, most plants in the 2020 field site bore copious amounts of honeydew at the start of the experiment, which may have been enough to satisfy local RIFA colony requirements (pers. observ.).

Compared to their initially high densities, a decrease in SA densities in 2020 may reflect a population bust. Aphids commonly cycle through population booms and busts, as mediated by bottom-up and top-down factors, such as plant health and natural enemy pressure, respectively

[239]. Nonetheless, because the initial 2019 aphid numbers were similar to the final 2020 aphid numbers, a RIFA-mediated boom in SAs may have occurred after the 2020 population bust. This would likely lead to RIFA recruitment and increases in RIFA-SA interactions. This hypothesis is supported by personal observations of less honeydew on plant leaves in 2020 at the end versus beginning of the experiment. Since RIFAs seek a honeydew reward, it is plausible that ants would actively tend and increase initially low SA populations, as they did in 2019.

RIFA Effects on SA Natural Enemies

A stronger correlation and response of parasitoid activity to increasing SA density in the presence versus absence of RIFA in 2019 is contrary to the expectation that ants would protect aphid colonies from parasitoid activity and suggests that RIFA does not significantly hinder parasitoid activity. This could be the result of behavioral modifications by parasitoids in the presence of RIFA. Parasitoids display behaviors facilitating successful oviposition while avoiding ant attack [240, 241]. Moreover, some parasitoids prefer ant-tended aphids because they are less defensive, and more likely to produce parasitoid offspring [242, 243]. As such, *A. nigritus* may benefit from RIFA presence by having a better selection of hosts relative to untended SAs. This hypothesis could be validated through field or laboratory observations of *A. nigritus* parasitism behavior in the presence and absence of RIFA. Nonetheless, the 2020 results showing a weak response of parasitoid activity to increasing aphid density in the presence versus absence of RIFA show that RIFA-mediation of parasitoid activity is variable. In contrast, the fact that RIFA had no effect on the response of predator activity to changes in SA density in 2019 or 2020 parallels previous findings of high predation pressure on SA in sorghum [56, 244, 245], and suggests that ants are not effectively impeding predator aggregative responses.

Roles of RIFA and Initial SA Density on Natural Enemy Activity

As with the behavioral modifications noted above, a stronger response of parasitoid activity to RIFA at initially low SA density in 2019 likely resulted from a RIFA-mediated rapid increase in available hosts. Since primary parasitoids are dependent on hosts for development, increases in host populations would naturally increase parasitoid densities. This would also explain a weaker parasitoid response in 2020, where initially high aphid numbers gradually decreased and limited the availability of hosts over time. Observations of a negative predator response to SA densities in 2020, irrespective of RIFA presence, align with Hewlett et al. [244], who found that predators effectively suppressed low-density populations of SA.

The SA-RIFA interaction may change in time, as observed in other ant-aphid mutualisms under greenhouse [229, 231, 246], and field conditions [247-249]. Recurring assessments of SA-RIFA interactions will allow the anticipation of any mutualism-mediated population increases of either pest [250, 251], and may help develop ways to manipulate the interaction for the benefit of growers. This work measuring the effects of red imported RIFA on SA population growth may serve as a baseline for future comparative studies of RIFA and SA interactions.

CHAPTER V

CONCLUSIONS

The grain sorghum agroecosystem is filled with several players that can help or hinder SA biological control. This dissertation assessed how SA interacts with both antagonists (parasitoids and predators) and potential mutualists (RIFAs) to determine the feasibility of natural enemy-mediated pest control. Regarding SA interactions with antagonists, I have confirmed the role of SA honeydew as an *A. nigritus* parasitoid attractant (Chapter II). Effects of SA honeydew on other parasitoids' attraction, as well as on their fitness is presently unexplored. This needs to be assessed to discern parasitoids most capable of attacking SAs while surviving on SA honeydew. Specifically, the effects of SA honeydew on parasitoid survival, longevity, and fertility—all prominent factors in parasitoid fitness [136, 252-254], should be studied. It may also be worth exploring the effects of honeydew on parasitoid sex ratios. Benelli et al. [255] hypothesize parasitoid diet to influence mating success and sperm viability, which could affect the proportion of female offspring within a population. While studies specifically linking diet to mating are limited [256, 257], effects of diet on sex ratios are well known. For instance, progeny of the leafroller parasitoid, *Dolichogenidea tasmanica,* are strongly male biased in the absence of floral resources [258]. In contrast, when provided a sucrose-based sugar diet, sex ratios of *Pteromalus cerealellae* offspring are female-biased [259]. Similarly, the egg parasitoid, *Trichogramma ostriniae*, produces significantly more females when fed honeydew from *Rhopalosiphum maidis* aphids [260]. Whether SA honeydew plays a role on altering parasitoid fitness, including SA parasitism rate, is unknown.

In addition to being a parasitoid attractant, SA honeydew is also preferred by *A. nigritus* when produced on Johnson grass, though it is unknown if Johnson grass honeydew is preferred over grain sorghum by other SA parasitoids. This is worth exploring, especially if *A. nigritus* host choices align with their honeydew preferences. Parasitoids have previously shown differential preferences for the same insect host feeding on grain sorghum versus Johnson grass. For example, a study by Baxendale et al. [261] showed a marked preference of *Tetrastichus* near *venustus* parasitoids for sorghum midge, *Cantarinia sarghicola*, on Johnson grass. In contrast, three other parasitoids, *Eupelmus papa*, *Tetrastichus* near *blaslophagi*, and *Aproslocelus diplosidis* preferred *C. sarghicola* when the midges fed on grain sorghum. Assessing the honeydew and host preferences of several parasitoids may help uncover the species most likely to attack overwintering SA in Johnson grass. In turn, populations of these parasitoids could be built up and released on Johnson grass to suppress SA numbers before grain sorghum is planted.

This dissertation also confirmed the possibility of fecundity compensation in SA when attacked by *A. nigritus* (Chapter III). Since many parasitoids probe aphids before accepting them as hosts, the fact that I observed a lack of compensation to simple wounding events is a positive result. Nonetheless, the results after successful parasitism could question the use of certain parasitoids in biological control programs. It is important to recognize that fecundity compensation was only assessed under *A. nigritus* parasitism in the parental and F_1 generations of SA. It is unknown if increases in reproduction extend to the F_2 or even F_3 generations, which could theoretically boost SA population growth and increase the baseline fecundity levels of future SA generations. Furthermore, this behavioral response remains to be tested in the field and with other SA parasitoids, notably *L. testaceipes*, the second most common SA parasitoid in College Station,

Texas [56]. *Aphelinus nigritus* effects on SA field populations need to be assessed to determine if compensation practically poses a threat to aphid control efforts. While this chapter mainly focuses on an SA parasitoid, the role of predators in SA reproductive strategies should also be highlighted. Even without direct contact, predators can induce aphid alarm pheromones that bring about aphid dispersal and other escaping behaviors [164]. For example, exposure to the 2,4,6-trimethylpyridine alkaloid from the pink-spotted lady beetle, *Coleomegilla maculata* induces strong avoidance behaviors in SA [262]. Given the results of this chapter, it is entirely possible that the presence of a nearby predator or predator volatile compounds may affect aphid reproduction, especially if the risk of death is high. A straightforward way to test this is by exposing SAs to predator volatiles as done by Zhou et al. [262], followed by observations of potential increases in reproduction.

It must be stressed that *A. nigritus* and other synovigenic parasitoids also host feed to obtain nutrients towards maturing eggs. Together, host feeding, and parasitism-related SA deaths could possibly offset and even minimize any reproductive compensation that increases SA numbers. The same is possible for SA predators who may eat as many aphids as they indirectly produce through any fecundity compensation they may trigger. As mentioned above, these possibilities should be explored through field studies exploring whether SA parasitism and predation produce fecundity compensation capable to impact SA populations levels at field scale. One other avenue worth exploring is whether parasitism induces SA fecundity compensation at different instars. In other aphids, it is already known that rates of parasitoid attack and time of aphid mummification differ by instar [80]. Aphid instars can also affect parasitoid handling and oviposition times because older instars tend to display stronger defensive or avoidance behaviors than younger

instars [263]. Though my experiments solely focused on adult aphids, earlier instars, who are less likely to avoid parasitism, may more readily allocate resources to increased rates of reproduction. SAs of all developmental stages are present in the field, and their reproductive responses to parasitism warrant attention.

This dissertation further confirms the existence of a RIFA-sorghum aphid mutualism (Chapter IV). However, the mutualism only operates at initially low aphid (15 aphids/leaf) densities where per capita RIFA tending rates are high. I hypothesize a weaker RIFA-sorghum aphid association due to the overabundance of honeydew nutrients that likely satisfy RIFA colony requirements. This is supported by the results, where initially high SA densities (60 aphids/leaf) did not increase in the presence of RIFAs. If the RIFA-sorghum aphid mutualism is food-dependent, which it appears to be, a mutualism-mediated SA population increase could be prevented by adding artificial sugar sources to fields. This type of sugar provisioning has successfully disrupted mutualisms before and promoted pest control by natural enemies [264-266]. RIFA sugar preferences are well studied [267-270], which makes the development of alternative sugar sources that disrupt mutualisms even more practical. Since the sugar composition of SA honeydew is already known, an artificial diet mimicking SA honeydew could be created to further dissociate RIFAs from linked SAs to a carbohydrate resource.

Interestingly, RIFA doesn't appear to disrupt natural enemy dynamics in this system. I observed a numerical response of both parasitoids and predators to RIFA-driven SA population increases, likely because more SAs were available to parasitize or consume. These results go against typical reports of RIFA as an aggressive tender who often attacks aphid natural enemies [79]. For

the purposes of SA biological control, RIFAs may not be aggressive SA tenders, which could make it easier to introduce or augment natural enemies in the field. Alternatively, RIFA-SA interactions may still be new, and require further assessments to determine their impact on SA antagonists. While this dissertation focused on RIFA-sorghum aphid interactions during the summer, when SA populations are highest, it is more so relevant to assess RIFA impacts on SA at the start of the sorghum growing season (mid-March). This is because biological control is often more effective when pest numbers are low [271-273]. If early season interactions between RIFAs and SAs do not hinder natural enemy growth, concerns over the negative effects of RIFAs could be mitigated in SA pest management.

Despite their small size and recent arrival, SAs have largely influenced the last 8 years of sorghum production in Texas. The fact that SA damage became widespread immediately after its 2013 introduction highlights the strength of biological invasions when a food source is abundant and natural enemy suppression is weak. The success of SA mirrors other invasive aphids who benefit from obligate parthenogenesis and can rapidly increase in population size [274]. This is helped by the general homogeneity of farm environments compared to natural landscapes, which favors aphid adaptation to specific food sources [275]. Nonetheless, the right application of natural enemies can impede aphid expansion if all possible factors influencing natural enemy-aphid interactions are considered.

The overarching message of this dissertation is to highlight the ecological complexity involved in the biocontrol of recently invasive species. For decades, pest management largely took a one-size-fits-all chemical approach while ignoring the idiosyncratic attributes of individual pests'

ecological interactions. While this led to short term successes, the development of insecticide resistance makes it continually pertinent to assess the biology of pest insects, their antagonists, and mutualists. This includes the often-overlooked ecological factors emphasized in this dissertation that, if widespread, can alter current pest control strategies against several aphid pests (invasive or native). Ideally, a sufficient understanding of pest ecology, paired with biological and chemical control under an integrated pest management framework should dampen effects of SA, as well as those of other aphids. As with cereal aphids before it, SA likely won't be the last pest introduced to a high-value crop. We should take advantage of this relatively new pest and consider all ecological factors that may apply to combatting future pest invasions.

REFERENCES

1. Van Emden, H.F. and R. Harrington, *Aphids as crop pests*. 2017: Cabi.
2. Ng, J.C. and K.L. Perry, *Transmission of plant viruses by aphid vectors.* Molecular plant pathology, 2004. **5**(5): p. 505-511.
3. Pimentel, D., et al., *Benefits and Costs of Pesticide Use in U. S. Food Production.* BioScience, 1978. **28**(12): p. 772-784.
4. Dewar, A.M., *The adverse impact of the neonicotinoid seed treatment ban on crop protection in oilseed rape in the United Kingdom.* Pest Management Science, 2017. **73**(7): p. 1305-1309.
5. Foster, S.P., et al., *A mutation (L1014F) in the voltage-gated sodium channel of the grain aphid, Sitobion avenae, is associated with resistance to pyrethroid insecticides.* Pest Management Science, 2014. **70**(8): p. 1249-1253.
6. Yuan, H.B., et al., *Lethal, sublethal and transgenerational effects of the novel chiral neonicotinoid pesticide cycloxaprid on demographic and behavioral traits of Aphis gossypii (Hemiptera: Aphididae).* Insect Science, 2017. **24**(5): p. 743-752.
7. Wang, K.Y., et al., *Resistance of Aphis gossypii (Homoptera : Aphididae) to selected insecticides on cotton from five cotton production regions in Shandong, China.* Journal of Pesticide Science, 2007. **32**(4): p. 372-378.
8. Altenbuchner, C., M. Larcher, and S. Vogel, *The impact of organic cotton cultivation on the livelihood of smallholder farmers in Meatu district, Tanzania.* Renewable Agriculture and Food Systems, 2016. **31**(1): p. 22-36.
9. Castañeda-Yslas, I.J., et al., *Biomonitoring with Micronuclei Test in Buccal Cells of Female Farmers and Children Exposed to Pesticides of Maneadero Agricultural Valley, Baja California, Mexico.* Journal of Toxicology, 2016.
10. Curwin, B., et al., *Pesticide use and practices in an Iowa farm family pesticide exposure study.* Journal of agricultural safety and health, 2002. **8**(4): p. 423.
11. Franco, F.C., et al., *Evaluating genotoxic risks in Brazilian public health agents occupationally exposed to pesticides: a multi-biomarker approach.* Environmental Science and Pollution Research, 2016. **23**(19): p. 19723-19734.
12. Lai, W.Y., *Pesticide use and health outcomes: Evidence from agricultural water pollution in China.* Journal of Environmental Economics and Management, 2017. **86**: p. 93-120.
13. Carazo-Rojas, E., et al., *Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem.* Environmental pollution, 2018. **241**: p. 800-809.
14. Tanaka, H. and T. Katagi, *Ecotoxicological risk assessment of pesticides in aquatic ecosystems.* R&D Report, 2008. **1**: p. 1-18.
15. Rossi, V., et al., *Critical success factors for the adoption of decision tools in IPM.* Agronomy, 2019. **9**(11): p. 710.
16. Anciso, J.R., et al., *IPM in Texas citrus.* Texas Cooperative Extension, The Texas A&M University System, Publication B-6121, 2002.
17. Bloem, S. and R.F. Mizell, *Tomato IPM in Florida.* EDIS, 2004. **2004**(8).
18. Reddy, C.G.V., *Integrated Management of Insect Pests on Canola and other Brassica Oilseed*. 2017.

19. DeBach, P. and D. Rosen, *Biological control by natural enemies*. 1991: CUP Archive.
20. Heinz, K.M. and J.M. Nelson, *Interspecific Interactions among Natural Enemies ofBemisiain an Inundative Biological Control Program.* Biological Control, 1996. **6**(3): p. 384-393.
21. Frank, S.D., *Biological control of arthropod pests using banker plant systems: Past progress and future directions.* Biological Control, 2010. **52**(1): p. 8-16.
22. Blaauw, B.R. and R. Isaacs, *Larger wildflower plantings increase natural enemy density, diversity, and biological control of sentinel prey, without increasing herbivore density.* Ecological Entomology, 2012. **37**(5): p. 386-394.
23. Grez, A.A., T. Zaviezo, and M.M. Gardiner, *Local predator composition and landscape affects biological control of aphids in alfalfa fields.* Biological Control, 2014. **76**: p. 1-9.
24. Bianchi, F.J., C.J. Booij, and T. Tscharntke, *Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control.* Proc Biol Sci, 2006. **273**(1595): p. 1715-27.
25. Birkhofer, K., et al., *Organic farming affects the biological control of hemipteran pests and yields in spring barley independent of landscape complexity.* Landscape Ecology, 2015. **31**(3): p. 567-579.
26. Boivin, G., T. Hance, and J. Brodeur, *Aphid parasitoids in biological control.* Canadian Journal of Plant Science, 2012. **92**(1): p. 1-12.
27. Brewer, M.J. and N.C. Elliott, *Biological control of cereal aphids in north america and mediating effects of host plant and habitat manipulations.* Annu Rev Entomol, 2004. **49**: p. 219-42.
28. Colares, F., et al., *Recruitment of aphidophagous arthropods to sorghum plants infested with Melanaphis sacchari and Schizaphis graminum (Hemiptera: Aphididae).* Biological Control, 2015. **90**: p. 16-24.
29. Pickett, C.H., et al., *Long term post-release impacts of the introduced parasitoid Peristenus relictus (Hymenoptera: Braconidae) on Lygus spp. (Hemiptera: Miridae) populations in California.* Biological Control, 2017. **114**: p. 30-38.
30. Hougardy, E. and N.J. Mills, *Factors influencing the abundance of Trioxys pallidus, a successful introduced biological control agent of walnut aphid in California.* Biological Control, 2009. **48**(1): p. 22-29.
31. Vandenbosch, R., et al., *BIOLOGICAL-CONTROL OF THE WALNUT APHID IN CALIFORNIA - IMPACT OF THE PARASITE, TRIOXYS-PALLIDUS.* Hilgardia, 1979. **47**(1): p. 1-13.
32. Pike, K.S., et al., *Host range and habitats of the aphid parasitoid Diaeretiella rapae (Hymenoptera : Aphidiidae) in Washington state.* Environmental Entomology, 1999. **28**(1): p. 61-71.
33. Koch, R.L. and T.L. Galvan, *Bad side of a good beetle: the North American experience with Harmonia axyridis*, in *From biological control to invasion: the ladybird Harmonia axyridis as a model species*. 2007, Springer. p. 23-35.
34. Howarth, F.G., *Non-target effects of biological control agents*, in *Biological control: measures of success*. 2000, Springer. p. 369-403.
35. Welch, K.D. and J.D. Harwood, *Temporal dynamics of natural enemy–pest interactions in a changing environment.* Biological control, 2014. **75**: p. 18-27.

36. Macfadyen, S., A.P. Davies, and M.P. Zalucki, *Assessing the impact of arthropod natural enemies on crop pests at the field scale.* Insect science, 2015. **22**(1): p. 20-34.
37. Afsheen, S., et al., *Differential attraction of parasitoids in relation to specificity of kairomones from herbivores and their by-products.* Insect Science, 2008. **15**(5): p. 381-397.
38. Kulikowski, A.J., *Ant–scale mutualism increases scale infestation, decreases folivory, and disrupts biological control in restored tropical forests.* Biotropica, 2020. **52**(4): p. 709-716.
39. Nibouche, S., et al., *Morphometric and molecular discrimination of the sugarcane aphid, Melanaphis sacchari,(Zehntner, 1897) and the sorghum aphid Melanaphis sorghi (Theobald, 1904).* PloS one, 2021. **16**(3): p. e0241881.
40. Pekarcik, A., *Biology and management of the sugarcane aphid, Melanaphis sacchari (Zehntner), a new pest of sorghum, Sorghum bicolor (L.), in Alabama.* 2016.
41. Pena-Martinez, R., et al., *Sexual forms of sorghum aphid complex Melanaphis sacchari/sorghi in Mexico.* Southwestern Entomologist, 2016. **41**(1): p. 127-132.
42. Knutson, A.B., Robert; Brewer,Michael; Bynum, Ed; Porter, Pat, *The Sugarcane Aphid: Management Guidelines for Grain and Forage Sorghum in Texas.* 2016, TAMU AgriLife Extension: Texas.
43. Villanueva, R., et al., *Sugarcane aphid: A new pest of sorghum.* Texas A&M AgriLife Extension, 2014.
44. Bowling, R.D., et al., *Sugarcane Aphid (Hemiptera: Aphididae): A New Pest on Sorghum in North America.* Journal of Integrated Pest Management, 2016. **7**(1): p. 12.
45. Eastop, V., *Notes on East African Aphids: VII—Grass and Cereal Stem-and Leaf-Feeding Species.* The East African Agricultural Journal, 1955. **20**(3): p. 209-212.
46. Blackman, R. and V. Eastop, *Aphids on the world crops*. 1984, Chichester: John Wiley and Sons.
47. Armstrong, J.S., et al., *Sugarcane aphid (Hemiptera: Aphididae): Host range and sorghum resistance including cross-resistance from greenbug sources.* Journal of economic entomology, 2015. **108**(2): p. 576-582.
48. Zapata, S.D., et al. *The Economic Impact of the Sugarcane Aphid On Sorghum Production*. in *2016 Annual Meeting, February 6-9, 2016, San Antonio, Texas*. 2016. Southern Agricultural Economics Association.
49. Knutson, A., et al., *The Sugarcane Aphid: A New Pest of Grain and Forage Sorghum. Texas A&M AgriLife Extension Service. Accessed December 21, 2015*. 2015.
50. Singh, B.U., *Biology and management of the sugarcane aphid, Melanaphis sacchari (Zehntner) (Homoptera: Aphididae), in sorghum: a review.* 2004. **23**(9): p. 739–755.
51. Mbulwe, L., et al., *Registration of Sorghum Germplasm Tx3408 and Tx3409 with Tolerance to Sugarcane Aphid [(Zehntner)].* Journal of Plant Registrations, 2016. **10**(1): p. 51-56.
52. Balikai, R. and S. Lingappa, *Bio-ecology and Management of Sorghum Aphid: Melanaphis Sacchari*. 2012: LAP Lambert Academic Publishing.
53. Zapata, S.D., et al., *Economic Impact of the Sugarcane Aphid Outbreak in South Texas.* Journal of Agricultural and Applied Economics, 2018. **50**(1): p. 104-128.
54. Zapata, S.D., et al., *Economic Impact of the Sugarcane Aphid Outbreak in South Texas.* Journal of Agricultural and Applied Economics, 2017: p. 1-25.

55. Brewer, M.J., et al., *Sugarcane aphid population growth, plant injury, and natural enemies on selected grain sorghum hybrids in Texas and Louisiana.* Journal of economic entomology, 2017. **110**(5): p. 2109-2118.
56. Maxson, E.L., et al., *Species composition and abundance of the natural enemies of sugarcane aphid, Melanaphis sacchari (Zehnter)(Hemiptera: Aphididae), on sorghum in Texas.* Proceedings of the Entomological Society of Washington, 2019. **121**(4): p. 657-680.
57. Budenberg, W., *Honeydew as a contact kairomone for aphid parasitoids.* Entomologia experimentalis et applicata, 1990. **55**(2): p. 139-148.
58. Tena, A., et al., *Parasitoid nutritional ecology in a community context: the importance of honeydew and implications for biological control.* Curr Opin Insect Sci, 2016. **14**: p. 100-4.
59. Wickremasinghe, M., *Searching behaviour of the aphid parasitoid Aphidius rhopalosiphi (Hymenoptera: Aphidiidae) in response to honeydew excreted by aphids on different host plants.* Ruhuna Journal of Science, 2012. **2**(1).
60. Hogervorst, P.A., F.L. Wäckers, and J. Romeis, *Effects of honeydew sugar composition on the longevity of Aphidius ervi.* Entomologia Experimentalis et Applicata, 2007. **122**(3): p. 223-232.
61. Banfield-Zanin, J.A., et al., *Predator mortality depends on whether its prey feeds on organic or conventionally fertilised plants.* Biological Control, 2012. **63**(1): p. 56-61.
62. Martinez, A.J., et al., *Aphid-encoded variability in susceptibility to a parasitoid.* Bmc Evolutionary Biology, 2014. **14**.
63. Ingerslew, K.S. and D.L. Finke, *Mechanisms Underlying the Nonconsumptive Effects of Parasitoid Wasps on Aphids.* Environmental Entomology, 2017. **46**(1): p. 75-83.
64. Barry, A. and K. Ohno, *Cornicle secretions of Uroleucon nigrotuberculatum (Homoptera: Aphididae) as the last bullet against lady beetle larvae.* Entomological Science, 2016. **19**(4): p. 410-415.
65. Battaglia, D., et al., *Cornicle secretion of Acyrthosiphon pisum (Homoptera: Aphididae) as a contact kairomone for the parasitoid Aphidius ervi (Hymenoptera: Braconidae).* European Journal of Entomology, 1993. **90**: p. 423-423.
66. Moayeri, H.R.S., A. Rasekh, and A. Enkegaard, *Influence of cornicle droplet secretions of the cabbage aphid, Brevicoryne brassicae, on parasitism behavior of naive and experienced Diaeretiella rapae.* Insect Science, 2014. **21**(1): p. 56-64.
67. Parvizi, Y., A. Rasekh, and J. Michaud, *Cornicle secretions by Aphis fabae (Hemiptera: Aphididae) result in age-dependent costs and improved host suitability for Lysiphlebus fabarum (Marshall)(Hymenoptera: Braconidae).* Bulletin of entomological research, 2017: p. 1-9.
68. Agabiti, B., R.J. Wassenaar, and L. Winder, *Dropping behaviour of pea aphid nymphs increases their development time and reduces their reproductive capacity as adults.* Peerj, 2016. **4**.
69. Meresman, Y., M. Ben-Ari, and M. Inbar, *Turning in mid-air allows aphids that flee the plant to avoid reaching the risky ground.* Integrative Zoology, 2017. **12**(5): p. 409-420.
70. Altincicek, B., J. Gross, and A. Vilcinskas, *Wounding-mediated gene expression and accelerated viviparous reproduction of the pea aphid Acyrthosiphon pisum.* Insect Mol Biol, 2008. **17**(6): p. 711-6.

71. Cardinale, B.J., et al., *Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem.* Ecology Letters, 2003. **6**(9): p. 857-865.
72. Yano, E., *Ecological considerations for biological control of aphids in protected culture.* Population Ecology, 2006. **48**(4): p. 333-339.
73. Tscharntke, T., et al., *Conservation biological control and enemy diversity on a landscape scale.* Biological Control, 2007. **43**(3): p. 294-309.
74. Sudd, J.H., *Ant aphid mutualism.* Aphids: their biology, natural enemies and control, 1987: p. 335-65.
75. Breton, L.M. and J.F. Addicott, *Density-dependent mutualism in an aphid-ant interaction.* Ecology, 1992. **73**(6): p. 2175-2180.
76. Völkl, W., et al., *Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences.* Oecologia, 1999. **118**(4): p. 483-491.
77. Offenberg, J., *Balancing between mutualism and exploitation: the symbiotic interaction between Lasius ants and aphids.* Behavioral Ecology and Sociobiology, 2001. **49**(4): p. 304-310.
78. Wilder, S.M., et al., *Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions.* Proceedings of the National Academy of Sciences, 2011. **108**(51): p. 20639-20644.
79. Vinson, S.B. and S.T. A. *Impact of the Imported Fire Ant on Laboratory Populations of Cotton Aphid (Aphis gossypii) Predators on JSTOR.* 2016; Available from: http://www.jstor.org/stable/3494974?seq=1#page_scan_tab_contents.
80. Wyckhuys, K.A.G., et al., *Potential exposure of a classical biological control agent of the soybean aphid, Aphis glycines, on non-target aphids in North America.* Biological Invasions, 2008. **11**(4): p. 857-871.
81. Tokunaga, E. and N. Suzuki, *Colony growth and dispersal in the ant-tended aphid, Aphis craccivora Koch, and the non-ant-tended aphid, Acyrthosiphon pisum Harris, under the absence of predators and ants.* Population ecology, 2008. **50**(1): p. 45-52.
82. Coppler, L.B., J.F. Murphy, and M.D. Eubanks, *Red Imported Fire Ants (Hymenoptera: Formicidae) Increase the Abundance of Aphids in Tomato.* Florida Entomologist, 2007. **90**(3): p. 419-425.
83. Kaplan, I. and M.D. Eubanks, *Disruption of Cotton Aphid (Homoptera: Aphididae)—Natural Enemy Dynamics by Red Imported Fire Ants (Hymenoptera: Formicidae).* 2002.
84. Liu, S.-s. and L.-h. Jiang, *Differential parasitism of Plutella xylostella (Lepidoptera: Plutellidae) larvae by the parasitoid Cotesia plutellae (Hymenoptera: Braconidae) on two host plant species.* Bulletin of Entomological Research, 2003. **93**(1): p. 65.
85. Lill, J., R. Marquis, and R. Ricklefs, *Host plants influence parasitism of forest caterpillars.* Nature, 2002. **417**(6885): p. 170-173.
86. Heard, S.B., et al., *On the elusiveness of enemy-free space: spatial, temporal, and host-plant-related variation in parasitoid attack rates on three gallmakers of goldenrods.* Oecologia, 2006. **150**(3): p. 421.
87. Elkhouly, A., et al., *Natural Abundance and Host Plant Preference of the Larval Pupal Endoparasitoid Opius pallipes Wesmail (Hymenoptera: Braconidae) on the Serpentine Leafminer Liriomyza trifolii (Burgess) on Some Summer Host Plants.* Asian Journal of Agricultural and Horticultural Research, 2018: p. 1-7.

88. Jeffries, M.J.L., J.H., *Enemy free space and the structure of ecological communities.* Biological Journal of Linnean Society, 1984.
89. Frechette, B., et al., *Do resistant plants provide an enemy-free space to aphids?* European Journal of Entomology, 2012. **109**(1).
90. Vosteen, I., J. Gershenzon, and G. Kunert, *Enemy-free space promotes maintenance of host races in an aphid species.* Oecologia, 2016. **181**(3): p. 659-672.
91. Holt, R.D. and J.H. Lawton, *Apparent competition and enemy-free space in insect host-parasitoid communities.* The American Naturalist, 1993. **142**(4): p. 623-645.
92. Gross, P., *Insect behavioral and morphological defenses against parasitoids.* Annual review of entomology, 1993. **38**(1): p. 251-273.
93. Lou, Y.g. and J.a. Cheng, *Host-recognition kairomone from Sogatella furcifera for the parasitoid Anagrus nilaparvatae.* Entomologia experimentalis et applicata, 2001. **101**(1): p. 59-67.
94. Lee, J.C., G.E. Heimpel, and G.L. Leibee, *Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp.* Entomologia experimentalis et applicata, 2004. **111**(3): p. 189-199.
95. Patt, J.M., G.C. Hamilton, and J.H. Lashomb, *Foraging success of parasitoid wasps on flowers: interplay of insect morphology, floral architecture and searching behavior.* Entomologia experimentalis et applicata, 1997. **83**(1): p. 21-30.
96. Lee, J.C., D.A. Andow, and G.E. Heimpel, *Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field.* Ecological Entomology, 2006. **31**(5): p. 470-480.
97. Woodring, J., et al., *Honeydew amino acids in relation to sugars and their role in the establishment of ant-attendance hierarchy in eight species of aphids feeding on tansy (Tanacetum vulgare).* Physiological Entomology, 2004. **29**(4): p. 311-319.
98. Byrne, D.N. and W.B. Miller, *Carbohydrate and amino acid composition of phloem sap and honeydew produced by Bemisia tabaci.* Journal of Insect Physiology, 1990. **36**(6): p. 433-439.
99. Yao, I. and S.I. Akimoto, *Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid Tuberculatus quercicola.* Ecological Entomology, 2002. **27**(6): p. 745-752.
100. Blüthgen, N., G. Gottsberger, and K. Fiedler, *Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest.* Austral Ecology, 2004. **29**(4): p. 418-429.
101. Leroy, P.D., et al., *Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies.* Nature communications, 2011. **2**: p. 348.
102. Faria, C.A., F.L. Wäckers, and T.C. Turlings, *The nutritional value of aphid honeydew for non-aphid parasitoids.* Basic and Applied Ecology, 2008. **9**(3): p. 286-297.
103. Wäckers, F.L., P.C. Van Rijn, and G.E. Heimpel, *Honeydew as a food source for natural enemies: making the best of a bad meal?* Biological Control, 2008. **45**(2): p. 176-184.
104. Shik, J.Z., A.D. Kay, and J. Silverman, *Aphid honeydew provides a nutritionally balanced resource for incipient Argentine ant mutualists.* Animal behaviour, 2014. **95**: p. 33-39.
105. Casas, J., et al., *Energy dynamics in a parasitoid foraging in the wild.* Journal of animal ecology, 2003. **72**(4): p. 691-697.

106. Vollhardt, I.M., et al., *Spatial distribution of flower vs. honeydew resources in cereal fields may affect aphid parasitism.* Biological Control, 2010. **53**(2): p. 204-213.
107. Strand, M.R. and S.B. Vinson, *Behavioral response of the parasitoid Cardiochiles nigriceps to a kairomone.* Entomologia experimentalis et applicata, 1982. **31**(2-3): p. 308-315.
108. Brown, R.L., et al., *Invasive Vespula wasps utilize kairomones to exploit honeydew produced by sooty scale insects, Ultracoelostoma.* Journal of chemical ecology, 2015. **41**(11): p. 1018-1027.
109. Mehrnejad, M.R. and M.J. Copland, *Behavioral responses of the parasitoid Psyllaephagus pistaciae (Hymenoptera: Encyrtidae) to host plant volatiles and honeydew.* Entomological science, 2006. **9**(1): p. 31-37.
110. Detrain, C., et al., *Aphid–ant mutualism: how honeydew sugars influence the behaviour of ant scouts.* Physiological Entomology, 2010. **35**(2): p. 168-174.
111. Zhou, A., et al., *Effects of tending by Solenopsis invicta (Hymenoptera: Formicidae) on the sugar composition and concentration in the honeydew of an invasive mealybug, Phenacoccus solenopsis (Hemiptera: Pseudococcidae).* Ethology, 2015. **121**(5): p. 492-500.
112. Hendrix, D.L., Y.-a. Wei, and J.E. Leggett, *Homopteran honeydew sugar composition is determined by both the insect and plant species.* Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 1992. **101**(1-2): p. 23-27.
113. Akbar, W., et al., *Feeding by sugarcane aphid, M elanaphis sacchari, on sugarcane cultivars with differential susceptibility and potential mechanism of resistance.* Entomologia Experimentalis et Applicata, 2014. **150**(1): p. 32-44.
114. Henter, H.J. and S. Via, *The Potential for coevolution in a host-parasitoid system .1. Genetic variation within an aphid population in susceptibility to a parasitic wasp.* Evolution, 1995. **49**(3): p. 427-438.
115. Hufbauer, R.A. and S. Via, *Evolution of an aphid-parasitoid interaction: Variation in resistance to parasitism among aphid populations specialized on different plants.* Evolution, 1999. **53**(5): p. 1435-1445.
116. Barbosa, P., et al., *Differential parasitism of macrolepidopteran herbivores on two deciduous tree species.* Ecology, 2001. **82**(3): p. 698-704.
117. EDDMapS, *Johnson Grass, Sorghum halepense Distribution*, in *Early Detection & Distribution Mapping System.* 2019, The University of Georgia - Center for Invasive Species and Ecosystem Health.
118. McWhorter, C., *Introduction and spread of johnsongrass in the United States.* Weed Science, 1971. **19**(5): p. 496-500.
119. Pringle, E.G., et al., *Plant-derived differences in the composition of aphid honeydew and their effects on colonies of aphid-tending ants.* Ecology and evolution, 2014. **4**(21): p. 4065-4079.
120. Sabri, A., et al., *Proteomic investigation of aphid honeydew reveals an unexpected diversity of proteins.* PloS one, 2013. **8**(9): p. e74656.
121. Zhou, A., et al., *Effect of host plants on honeydew production of an invasive mealybug, Phenacoccus solenopsis (Hemiptera: Pseudococcidae).* Journal of insect behavior, 2013. **26**(2): p. 191-199.

122. Longley, M. and P.C. Jepson, *Effects of honeydew and insecticide residues on the distribution of foraging aphid parasitoids under glasshouse and field conditions.* Entomologia Experimentalis et Applicata, 1996. **81**(2): p. 189-198.
123. Steppuhn, A. and F. Wäckers, *HPLC sugar analysis reveals the nutritional state and the feeding history of parasitoids.* Functional Ecology, 2004. **18**(6): p. 812-819.
124. Oksanen, J., et al., *Package 'vegan'.* Community ecology package, version, 2013. **2**(9): p. 1-295.
125. Clavijo Mccormick, A., J. Gershenzon, and S.B. Unsicker, *Little peaks with big effects: establishing the role of minor plant volatiles in plant–insect interactions.* Plant, cell & environment, 2014. **37**(8): p. 1836-1844.
126. Friard, O.P. and M. Gamba, *Behavioral Observation Research Interactive Software (BORIS).* 2016.
127. Shaaban, B., et al., *Sugar, amino acid and inorganic ion profiling of the honeydew from different hemipteran species feeding on Abies alba and Picea abies.* PloS one, 2020. **15**(1): p. e0228171.
128. Brinza, L., et al., *Systemic analysis of the symbiotic function of Buchnera aphidicola, the primary endosymbiont of the pea aphid Acyrthosiphon pisum.* Comptes rendus biologies, 2009. **332**(11): p. 1034-1049.
129. Mohammed, N.A., I.A.M. Ahmed, and E.E. Babiker, *Nutritional evaluation of sorghum flour (Sorghum bicolor L. Moench) during processing of injera.* World Academy of Science, Engineering and Technology, 2011. **51**: p. 72-76.
130. Ajakaiye, C.O., *Amino acid composition of sorghum grains as influenced by grain maturity, genotype, and nitrogen fertilization.* Journal of Agricultural and Food Chemistry, 1984. **32**(1): p. 47-50.
131. McWhorter, C., *Water-soluble carbohydrates in johnsongrass.* Weed Science, 1974: p. 159-163.
132. Horowitz, M., *Seasonal development of established johnsongrass.* Weed Science, 1972: p. 392-395.
133. Neucere, N.J. and G. Sumrell, *Chemical composition of different varieties of grain sorghum.* Journal of agricultural and food chemistry, 1980. **28**(1): p. 19-21.
134. Shimron, O., A. Hefetz, and D. Gerling, *Arrestment responses of Eretmocerus species and Encarsia deserti (Hymenoptera: Aphelinidae) to Bemisia tabaci honeydew.* Journal of insect behavior, 1992. **5**(4): p. 517-526.
135. Romeis, J. and C. Zebitz, *Searching behaviour of Encarsia formosa as mediated by colour and honeydew.* Entomologia Experimentalis et Applicata, 1997. **82**(3): p. 299-309.
136. Hirose, Y., et al., *Effects of sugars on the longevity of adult females of Eretmocerus eremicus and Encarsia formosa (Hymenoptera: Aphelinidae), parasitoids of Bemisia tabaci and Trialeurodes vaporariorum (Hemiptera: Alyerodidae), as related to their honeydew feeding and host feeding.* Applied entomology and zoology, 2009. **44**(1): p. 175-181.
137. Wäckers, F.L., *Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars.* Oikos, 2000. **90**(1): p. 197-201.

138. Vollhardt, I.M., et al., *Nectar vs. honeydew feeding by aphid parasitoids: does it pay to have a discriminating palate?* Entomologia experimentalis et applicata, 2010. **137**(1): p. 1-10.
139. Lewis, W., et al., *Understanding how parasitoids balance food and host needs: importance to biological control.* Biological control, 1998. **11**(2): p. 175-183.
140. Křivan, V. and E. Sirot, *Searching for food or hosts: the influence of parasitoids behavior on host–parasitoid dynamics.* Theoretical Population Biology, 1997. **51**(3): p. 201-209.
141. Heimpel, G., et al., *Gut sugar analysis in field-caught parasitoids: adapting methods originally developed for biting flies.* International Journal of Pest Management, 2004. **50**(3): p. 193-198.
142. Zhang, Y., et al., *Effect of six carbohydrate sources on the longevity of a whitefly parasitoid Eretmocerus hayati (Hymenoptera: Aphelinidae).* Journal of Asia-Pacific Entomology, 2014. **17**(4): p. 723-728.
143. Jervis, M.A., J. Ellers, and J.A. Harvey, *Resource acquisition, allocation, and utilization in parasitoid reproductive strategies.* Annu. Rev. Entomol., 2008. **53**: p. 361-385.
144. McWHORTER, C.G., R.N. Paul, and J.C. Ouzts, *Bicellular trichomes of johnsongrass (Sorghum halepense) leaves: morphology, histochemistry, and function.* Weed Science, 1995: p. 201-208.
145. Mulatu, B., S.W. Applebaum, and M. Coll, *Effect of tomato leaf traits on the potato tuber moth and its predominant larval parasitoid: a mechanism for enemy-free space.* Biological Control, 2006. **37**(2): p. 231-236.
146. Keller, M.A., *Influence of leaf surfaces on movements by the hymenopterous parasitoid Trichogramma exiguum.* Entomologia Experimentalis et Applicata, 1987. **43**(1): p. 55-59.
147. Gols, R., et al., *Are population differences in plant quality reflected in the preference and performance of two endoparasitoid wasps?* Oikos, 2009. **118**(5): p. 733-742.
148. Reznick, D., *Costs of reproduction: an evaluation of the empirical evidence.* Oikos, 1985: p. 257-267.
149. Hopper, K.R., *Preference, acceptance, and fitness components of Microplitis croceipes (Hymenoptera: Braconidae) attacking various instars of Heliothis virescens (Lepidoptera: Noctuidae).* Environmental Entomology, 1986. **15**(2): p. 274-280.
150. Eben, A., et al., *Host species and host plant effects on preference and performance of Diachasmimorpha longicaudata (Hymenoptera: Braconidae).* Environmental Entomology, 2000. **29**(1): p. 87-94.
151. Traugott, M., et al., *Generalist predators disrupt parasitoid aphid control by direct and coincidental intraguild predation.* Bulletin of Entomological Research, 2012. **102**(2): p. 239-247.
152. Velasco-Hernández, M.C., et al., *Intraguild predation on the whitefly parasitoid Eretmocerus eremicus by the generalist predator Geocoris punctipes: a behavioral approach.* PloS one, 2013. **8**(11): p. e80679.
153. Xue, Y., et al., *Intraguild predation of the aphid parasitoid Aphelinus certus by Coccinella septempunctata and Harmonia axyridis.* BioControl, 2012. **57**(5): p. 627-634.
154. Meisner, M., et al., *Intraguild predation on the parasitoid Aphidius ervi by the generalist predator Harmonia axyridis: the threat and its avoidance.* Entomologia Experimentalis et Applicata, 2011. **138**(3): p. 193-201.

155. Nakashima, Y., et al., *Chemically mediated intraguild predator avoidance by aphid parasitoids: interspecific variability in sensitivity to semiochemical trails of ladybird predators.* Journal of Chemical Ecology, 2006. **32**(9): p. 1989-1998.
156. Fischer, C.Y., et al., *Bacteria may enhance species association in an ant–aphid mutualistic relationship.* Chemoecology, 2015. **25**(5): p. 223-232.
157. Peach, D.A., et al., *Attraction of female Aedes aegypti (L.) to aphid honeydew*. Insects, 2019. **10**(2): p. 43.
158. Sanchéz, E.R., et al., *Biological activity of Bacillus thuringiensis culture supernatant on Bemisia tabaci and its parasitoid Eretmocerus eremicus.* Tropical and Subtropical Agroecosystems, 2019. **22**(1).
159. Fand, B.B., et al., *Bacterial volatiles from mealybug honeydew exhibit kairomonal activity toward solitary endoparasitoid Anagyrus dactylopii.* Journal of Pest Science, 2020. **93**(1): p. 195-206.
160. Ingerslew, K.S. and D.L. Finke, *Multi-species suppression of herbivores through consumptive and non-consumptive effects.* PloS one, 2018. **13**(5): p. e0197230.
161. Werner, E.E. and S.D. Peacor, *A review of trait-mediated indirect interactions in ecological communities.* Ecology, 2003. **84**(5): p. 1083-1100.
162. Desneux, N., et al., *Multifaceted determinants of host specificity in an aphid parasitoid.* Oecologia, 2009. **160**(2): p. 387-398.
163. Hopper, K.R., et al., *Host specificity of Aphelinus species collected from soybean aphid in Asia.* Biological Control, 2017. **115**: p. 55-73.
164. Vandermoten, S., et al., *Aphid alarm pheromone: an overview of current knowledge on biosynthesis and functions.* Insect biochemistry and molecular biology, 2012. **42**(3): p. 155-163.
165. De Farias, A.M. and K.R. Hopper, *Oviposition behavior of Aphelinus asychis (Hymenoptera: Aphelinidae) and Aphidius matricariae (Hymenoptera: Aphidiidae) and defense behavior of their host Diuraphis noxia (Homoptera: Aphididae).* Environmental entomology, 1999. **28**(5): p. 858-862.
166. Micha, S.G. and U. Wyss, *Aphid alarm pheromone (E)-β-farnesene: a host finding kairomone for the aphid primary parasitoid Aphidius uzbekistanicus (Hymenoptera: Aphidiinae).* Chemoecology, 1996. **7**(3): p. 132-139.
167. Vosteen, I., W.W. Weisser, and G. Kunert, *Is there any evidence that aphid alarm pheromones work as prey and host finding kairomones for natural enemies?* Ecological Entomology, 2016. **41**(1): p. 1-12.
168. Oliver, K.M., N.A. Moran, and M.S. Hunter, *Variation in resistance to parasitism in aphids is due to symbionts not host genotype.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(36): p. 12795-12800.
169. Renault, S., et al., *Effects of the Diadromus pulchellus ascovirus, DpAV-4, on the hemocytic encapsulation response and capsule melanization of the leek-moth pupa, Acrolepiopsis assectella.* Journal of Insect Physiology, 2002. **48**(3): p. 297-302.
170. Gwynn, D., et al., *Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid.* Proceedings of the Royal Society of London B: Biological Sciences, 2005. **272**(1574): p. 1803-1808.
171. Vale, P. and T. Little, *Fecundity compensation and tolerance to a sterilizing pathogen in Daphnia.* Journal of evolutionary biology, 2012. **25**(9): p. 1888-1896.

172. Abram, P.K., et al., *Nonreproductive Effects of Insect Parasitoids on Their Hosts.* Annual review of entomology, 2019. **64**: p. 259-276.
173. Giordanengo, P. and J. Nenon, *Melanization and encapsulation of eggs and larvae of Epidinocarsis lopezi by its host Phenacoccus manihoti; effects of superparasitism and egg laying patterns.* Entomologia experimentalis et applicata, 1990. **56**(2): p. 155-163.
174. Suma, P., et al., *Encapsulation rates of the parasitoid Anagyrus sp. nr. pseudococci, by three mealybug species (Hemiptera: Pseudococcidae).* Phytoparasitica, 2012. **40**(1): p. 11-16.
175. Coors, A. and L. De Meester, *Synergistic, antagonistic and additive effects of multiple stressors: predation threat, parasitism and pesticide exposure in Daphnia magna.* Journal of Applied Ecology, 2008. **45**(6): p. 1820-1828.
176. Slos, S. and R. Stoks, *Predation risk induces stress proteins and reduces antioxidant defense.* Functional Ecology, 2008. **22**(4): p. 637-642.
177. McCauley, S.J., L. Rowe, and M.-J. Fortin, *The deadly effects of "nonlethal" predators.* Ecology, 2011. **92**(11): p. 2043-2048.
178. Martinez, A.J., et al., *Multi-modal defences in aphids offer redundant protection and increased costs likely impeding a protective mutualism.* Journal of Animal Ecology, 2018. **87**(2): p. 464-477.
179. Laughton, A.M., et al., *Characterisation of immune responses in the pea aphid, Acyrthosiphon pisum.* Journal of insect physiology, 2011. **57**(6): p. 830-839.
180. Martinez, A.J., et al., *Specificity of multi-modal aphid defenses against two rival parasitoids.* PloS one, 2016. **11**(5): p. e0154670.
181. Cayetano, L. and C. Vorburger, *Symbiont-conferred protection against Hymenopteran parasitoids in aphids: how general is it?* Ecological Entomology, 2015. **40**(1): p. 85-93.
182. Agnew, P., J.C. Koella, and Y. Michalakis, *Host life history responses to parasitism.* Microbes and Infection, 2000. **2**(8): p. 891-896.
183. Yongzhen, P., L. Shuping, and L. Changguo, *Effect of delay on a predator–prey model with parasitic infection.* Nonlinear Dynamics, 2011. **63**(3): p. 311-321.
184. Polak, M. and W.T. Starmer, *Parasite–induced risk of mortality elevates reproductive effort in male Drosophila.* Proceedings of the Royal Society of London. Series B: Biological Sciences, 1998. **265**(1411): p. 2197-2201.
185. Shoemaker, K., N. Parsons, and S. Adamo, *Egg-laying behaviour following infection in the cricket Gryllus texensis.* Canadian journal of zoology, 2006. **84**(3): p. 412-418.
186. Chadwick, W. and T.J. Little, *A parasite-mediated life-history shift in Daphnia magna.* Proceedings of the Royal Society of London B: Biological Sciences, 2005. **272**(1562): p. 505-509.
187. Leventhal, G.E., R.P. Dunner, and S.M. Barribeau, *Delayed Virulence and Limited Costs Promote Fecundity Compensation upon Infection.* American Naturalist, 2014. **183**(4): p. 480-493.
188. Kaiser, M.C. and G.E. Heimpel, *Parasitoid-induced transgenerational fecundity compensation in an aphid.* Entomologia Experimentalis Et Applicata, 2016. **159**(2): p. 197-206.
189. Kaiser, M., *Transgenerational Fecundity Compensation And Post-Parasitism Reproduction By Aphids In Response To Their Parasitoids.* 2017.

190. Holt, J.R., et al., *Differences in Microbiota between two Multilocus Lineages of the sugarcane Aphid (Melanaphis sacchari) in the continental United States.* Annals of the Entomological Society of America, 2020. **113**(4): p. 257-265.
191. Agarwala, B.K. and P.R. Choudhury, *Host races of the cotton aphid, Aphis gossypii, in asexual populations from wild plants of taro and brinjal.* Journal of Insect Science, 2013. **13**(1): p. 34.
192. Hopper, K.R., et al., *Host specificity of Aphelinus species considered for introduction to control Diuraphis noxia.* Biological Control, 2017. **107**: p. 21-32.
193. Vorburger, C., et al., *Limited scope for maternal effects in aphid defence against parasitoids.* Ecological Entomology, 2008. **33**(2): p. 189-196.
194. Fox, C.W. and T.A. Mousseau, *Maternal effects as adaptations for transgenerational phenotypic plasticity in insects.* Maternal effects as adaptations, 1998. **159**(1998): p. 159-177.
195. Moret, Y., *'Trans-generational immune priming': specific enhancement of the antimicrobial immune response in the mealworm beetle, Tenebrio molitor.* Proceedings of the Royal Society B: Biological Sciences, 2006. **273**(1592): p. 1399-1405.
196. Tegelaar, K. and O. Leimar, *Alate production in an aphid in relation to ant tending and alarm pheromone.* Ecological Entomology, 2014. **39**(5): p. 664-666.
197. Hodgson, E., et al., *Alate production of soybean aphid (Homoptera: Aphididae) in Minnesota.* Environmental Entomology, 2005. **34**(6): p. 1456-1463.
198. Tetreau, G., et al., *Trans-generational immune priming in invertebrates: current knowledge and future prospects.* Frontiers in Immunology, 2019. **10**: p. 1938.
199. Moreau, J., et al., *Trans-generational immune priming is constrained by the maternal immune response in an insect.* Oikos, 2012. **121**(11): p. 1828-1832.
200. Dubuffet, A., et al., *Trans-generational immune priming protects the eggs only against gram-positive bacteria in the mealworm beetle.* PLoS Pathog, 2015. **11**(10): p. e1005178.
201. Schneider, D.S. and J.S. Ayres, *Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases.* Nature Reviews Immunology, 2008. **8**(11): p. 889-895.
202. Best, A., A. White, and M. Boots, *Maintenance of host variation in tolerance to pathogens and parasites.* Proceedings of the National Academy of Sciences, 2008. **105**(52): p. 20786-20791.
203. Best, A., A. White, and M. Boots, *Resistance is futile but tolerance can explain why parasites do not always castrate their hosts.* Evolution: International Journal of Organic Evolution, 2010. **64**(2): p. 348-357.
204. Barribeau, S., D. Sok, and N. Gerardo, *Aphid reproductive investment in response to mortality risks.* Bmc Evolutionary Biology, 2010. **10**.
205. Digilio, M.C., et al., *Host castration by Aphidius ervi venom proteins.* Journal of insect Physiology, 2000. **46**(6): p. 1041-1050.
206. Colinet, D., et al., *Identification of the main venom protein components of Aphidius ervi, a parasitoid wasp of the aphid model Acyrthosiphon pisum.* BMC genomics, 2014. **15**(1): p. 1-14.
207. Cloutier, C. and A.E. Douglas, *Impact of a parasitoid on the bacterial symbiosis of its aphid host.* Entomologia Experimentalis et Applicata, 2003. **109**(1): p. 13-19.

208. Pennacchio, F. and D. Mancini, *Aphid parasitoid venom and its role in host regulation*, in *Parasitoid Viruses*. 2012, Elsevier. p. 247-254.
209. Falabella, P., et al., *A γ-glutamyl transpeptidase of Aphidius ervi venom induces apoptosis in the ovaries of host aphids.* Insect Biochemistry and Molecular Biology, 2007. **37**(5): p. 453-465.
210. Copland, M., *Female reproductive system of the Aphelinidae (Hymenoptera: Chalcidoidea).* International Journal of Insect Morphology and Embryology, 1976. **5**(3): p. 151-166.
211. Mancini, D., A.P. Garonna, and P.A. Pedata, *To divide or not to divide: An alternative behavior for teratocytes in Encarsia pergandiella (Hymenoptera: Aphelinidae).* Arthropod structure & development, 2016. **45**(1): p. 57-63.
212. Cônsoli, F., et al., *In vitro culture of the teratocytes of Trissolcus basalis (Hymenoptera, Scelionidae) and their requirements for host-derived components.* Biological Control, 2001. **22**(2): p. 176-184.
213. Cusumano, A., et al., *First extensive characterization of the venom gland from an egg parasitoid: Structure, transcriptome and functional role.* Journal of insect physiology, 2018. **107**: p. 68-80.
214. Mercer, N.H., R.T. Bessin, and J.J. Obrycki, *Parasitization of the sugarcane aphid, Melanaphis sacchari, by commercially available aphid parasitoids.* BioControl, 2020: p. 1-11.
215. Hung, A.C. and S.B. Vinson, *Factors affecting the distribution of fire ants in Texas (Myrmicinae: Formicidae).* The Southwestern Naturalist, 1978: p. 205-213.
216. Collins, L. and R.H. Scheffrahn, *Red Imported Fire Ant, Solenopsis invicta Buren (Insecta: Hymenoptera: Formicidae: Myrmicinae)*. 2001: University of Florida Cooperative Extension Service, Institute of Food and ….
217. Diaz, R., A. Knutson, and J.S. Bernal, *Effect of the Red Imported Fire Ant on Cotton Aphid Population Density and Predation of Bollworm and Beet Armyworm Eggs.* Journal of Economic Entomology, 2004. **97**(2): p. 222-229.
218. Sharma, S., D.H. Oi, and E.A. Buss, *Honeydew-producing hemipterans in Florida associated with Nylanderia fulva (Hymenoptera: Formicidae), an invasive crazy ant.* Florida Entomologist, 2013. **96**(2): p. 538-547.
219. Wilder, S.M., et al., *Introduced fire ants can exclude native ants from critical mutualist-provided resources.* Oecologia, 2013. **172**(1): p. 197-205.
220. Zenner-Polanía, I. and O. Martínez, *Impacto ecologico de la hormiga loca, Paratrechina fulva (Mayr), en el municipio de Cimitarra (Santander).* Revista Colombiana de Entomología, 1992. **18**(1): p. 14-22.
221. Zhou, A., et al., *Does mutualism drive the invasion of two alien species? The case of Solenopsis invicta and Phenacoccus solenopsis.* PloS one, 2012. **7**(7): p. e41856.
222. Wylie, F.R. and S. Janssen-May, *Red imported fire ant in Australia: what if we lose the war?* Ecological Management & Restoration, 2017. **18**(1): p. 32-44.
223. Jetter, K., J. Hamilton, and J. Klotz, *Eradication costs calculated: Red imported fire ants threaten agriculture, wildlife and homes.* California Agriculture, 2002. **56**(1): p. 26-34.
224. Kaplan, I. and M.D. Eubanks, *Aphids alter the community-wide impact of fire ants.* Ecology, 2005. **86**(6): p. 1640-1649.

225. Hall, D.G., *The sugarcane aphid, Melanaphis sacchari (Zehntner), in Florida.* Journal of the American Society Sugar Cane Technologists, 1987. **7**: p. 26-29.
226. White, W., T. Reagan, and D. Hall, *Melanaphis sacchari (Homoptera: Aphididae), a sugarcane pest new to Louisiana.* Florida Entomologist, 2001. **84**(3): p. 435-436.
227. Hall, K.D., et al., *The progressive increase of food waste in America and its environmental impact.* PloS one, 2009. **4**(11): p. e7940.
228. Calabuig, A., F. Garcia-Marí, and A. Pekas, *Ants in citrus: impact on the abundance, species richness, diversity and community structure of predators and parasitoids.* Agriculture, Ecosystems & Environment, 2015. **213**: p. 178-185.
229. Canedo-Júnior, E., et al., *The effect size of aphid-tending ants in an agricultural tri-trophic system.* Journal of Applied Entomology, 2018. **142**(3): p. 349-358.
230. Morales, M.A., *Mechanisms and density dependence of benefit in an ant–membracid mutualism.* Ecology, 2000. **81**(2): p. 482-489.
231. Schwartzberg, E.G., D. Johnson, and G. Brown, *The influence of Lasius neoniger (Hymenoptera: Formicidae) on population growth and biomass of Aphis glycines (Hemiptera: Aphididae) in soybeans.* Environmental entomology, 2010. **39**(6): p. 1788-1793.
232. Yang, Y., et al., *Sugarcane Aphid (Hemiptera: Aphididae) on Sorghum. I. Population Characteristics and Dispersion Patterns in Relation to Different Sample Unit Sizes.* Environmental Entomology, 2021. **50**(2): p. 489-503.
233. Styrsky, J.D. and M.D. Eubanks, *Ecological consequences of interactions between ants and honeydew-producing insects.* Proceedings of the Royal Society of London B: Biological Sciences, 2007. **274**(1607): p. 151-164.
234. Zhang, S., Y. Zhang, and K. Ma, *Mixed effects of ant–aphid mutualism on plants across different spatial scales.* Basic and Applied Ecology, 2015. **16**(5): p. 452-459.
235. Knutson, A.E. and M. Campos, *Effect of red imported fire ant, Solenopsis invicta1, on abundance of corn earworm, Helicoverpa zea2, on maize in Texas.* Southwestern Entomologist, 2008. **33**(1): p. 1-13.
236. Daane, K.M., et al., *Effects of liquid insecticide baits on Argentine ants in California's coastal vineyards.* Crop Protection, 2006. **25**(6): p. 592-603.
237. Teetes, G.L., E.G. Lopez, and C.A. Schaefer, *Seasonal Abundance of the Greenbug and its Natural Enemies in Grain Sorghum in the Texas High Plains.* Texas FARMER Collection, 1975.
238. Addicott, J.F., *A multispecies aphid–ant association: density dependence and species-specific effects.* Canadian Journal of Zoology, 1979. **57**(3): p. 558-569.
239. Matis, J.H., et al., *Comparisons of Two Symmetric Density Function Solutions of Aphid Population Growth Models.* 2008.
240. Daane, K.M., et al., *Impacts of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards.* Ecological Entomology, 2007. **32**(6): p. 583-596.
241. Völkl, W., *Parasitoid learning during interactions with ants: how to deal with an aggressive antagonist.* Behavioral Ecology and Sociobiology, 2001. **49**(2-3): p. 135-144.
242. Völkl, W., *The effect of ant-attendance on the foraging behaviour of the aphid parasitoid Lysiphlebus cardui.* Oikos, 1994: p. 149-155.
243. Rasekh, A., et al., *Ant mimicry by an aphid parasitoid, Lysiphlebus fabarum.* Journal of Insect Science, 2010. **10**(1).

244. Hewlett, J.A., A. Szczepaniec, and M.D. Eubanks, *The effects of sugarcane aphid density in sorghum on predation by lady beetles and lacewings.* Biological Control, 2019. **129**: p. 171-177.
245. Karabo, O., M. Obopile, and B. Tiroesele, *Insect diversity and population dynamics of natural enemies under sorghum–legume intercrops.* Transactions of the Royal Society of South Africa, 2019. **74**(3): p. 258-267.
246. Hosseini, A., et al., *Effect of ant attendance on aphid population growth and above ground biomass of the aphid's host plant.* European Journal of Entomology, 2017. **114**: p. 106.
247. Kindlmann, P., M. Hullé, and B. Stadler, *Timing of dispersal: effect of ants on aphids.* Oecologia, 2007. **152**(4): p. 625-631.
248. Miñarro, M., Fernández-Mata., and P. Medina, *Role of ants in structuring the aphid community on apple.* Ecological Entomology, 2010. **35**(2): p. 206-215.
249. Wu, D., et al., *Effects of Solenopsis invicta (Hymenoptera: Formicidae) and its interaction with aphids on the seed productions of mungbean and rapeseed plants.* Journal of Economic Entomology, 2014. **107**(5): p. 1758-1764.
250. Nagy, C., J.V. Cross, and V. Markó, *Can artificial nectaries outcompete aphids in ant-aphid mutualism? Applying artificial sugar sources for ants to support better biological control of rosy apple aphid, Dysaphis plantaginea Passerini in apple orchards.* Crop Protection, 2015. **77**: p. 127-138.
251. Kaneko, S., *Seasonal differences in the abundance of an ant-adapted parasitoid, Lysiphlebus japonicus (Hymenoptera: Aphidiidae), in ant-attended colonies of the spirea aphid Aphis spiraecola (Hemiptera: Aphididae) on citrus: comparison for two ant species, Lasius japonicus and Pristomyrmex punctatus (Hymenoptera: Formicidae).* Applied entomology and zoology, 2018. **53**(3): p. 315-321.
252. Dulaurent, A.M., et al., *Honeydew feeding increased the longevity of two egg parasitoids of the pine processionary moth.* Journal of Applied Entomology, 2011. **135**(3): p. 184-194.
253. Hagenbucher, S., F.L. Wäckers, and J. Romeis, *Aphid honeydew quality as a food source for parasitoids is maintained in Bt cotton.* PloS one, 2014. **9**(9): p. e107806.
254. Tena, A., et al., *The influence of aphid-produced honeydew on parasitoid fitness and nutritional state: A comparative study.* Basic and Applied Ecology, 2018. **29**: p. 55-68.
255. Benelli, G., et al., *The impact of adult diet on parasitoid reproductive performance.* Journal of Pest Science, 2017. **90**(3): p. 807-823.
256. Blaul, B. and J. Ruther, *How parasitoid females produce sexy sons: a causal link between oviposition preference, dietary lipids and mate choice in Nasonia.* Proceedings of the Royal Society B: Biological Sciences, 2011. **278**(1722): p. 3286-3293.
257. Cotton, S., K. Fowler, and A. Pomiankowski, *Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis?* Proceedings of the Royal Society of London. Series B: Biological Sciences, 2004. **271**(1541): p. 771-783.
258. Berndt, L.A. and S.D. Wratten, *Effects of alyssum flowers on the longevity, fecundity, and sex ratio of the leafroller parasitoid Dolichogenidea tasmanica.* Biological Control, 2005. **32**(1): p. 65-69.

259. Onagbola, E.O., H.Y. Fadamiro, and G.N. Mbata, *Longevity, fecundity, and progeny sex ratio of Pteromalus cerealellae in relation to diet, host provision, and mating.* Biological Control, 2007. **40**(2): p. 222-229.
260. Fuchsberg, J.R., et al., *Evaluation of corn leaf aphid (Rhopalosiphum maidis; Homoptera: Aphididae) honeydew as a food source for the egg parasitoid Trichogramma ostriniae (Hymenoptera: Trichogrammatidae).* Biological Control, 2007. **40**(2): p. 230-236.
261. Baxendale, F.P., C. Lippincott, and G. Teetes, *Biology and seasonal abundance of hymenopterous parasitoids of sorghum midge (Diptera: Cecidomyiidae).* Environmental Entomology, 1983. **12**(3): p. 871-877.
262. Zhou, A., et al., *Behavioral and electrophysiological response of sugarcane aphid and imported fire ant to 2,4,6-trimethylpyridine, an alkaloid from the pink-spotted lady beetle.* Journal of Pest Science, 2021.
263. Chau, A. and M. Mackauer, *Host-instar selection in the aphid parasitoid Monoctonus paulensis (Hymenoptera: Braconidae, Aphidiinae): a preference for small pea aphids.* European Journal of Entomology, 2000. **97**(3): p. 347-354.
264. Tena, A., et al., *Sugar provisioning maximizes the biocontrol service of parasitoids.* Journal of Applied Ecology, 2015. **52**(3): p. 795-804.
265. Pérez-Rodríguez, J., et al., *Sugar provisioning for ants enhances biological control of mealybugs in citrus.* Biological Control, 2021. **157**: p. 104573.
266. Wäckers, F.L., et al., *Attract and distract: Manipulation of a food-mediated protective mutualism enhances natural pest control.* Agriculture, Ecosystems & Environment, 2017. **246**: p. 168-174.
267. Drees, B.M., *Collecting and Maintaining Colonies of Red Imported Fire Ants for Study*. 2012, TAMU AgriLife Extension.
268. Agnew, C.W., W.L. Sterling, and D.A. Dean, *Influence of Cotton Nectar on Red Imported Fire Ants and Other Predators.* 1982.
269. Lanza, J., et al., *Preferences of the fire ants Solenopsis invicta and S. geminata (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectars.* Environmental Entomology, 1993. **22**(2): p. 411-417.
270. Glunn, F., D.F. Howard, and W.R. Tschinkel, *Food preference in colonies of the fire antSolenopsis invicta.* Insectes Sociaux, 1981. **28**(2): p. 217-222.
271. Sigsgaard, L., *A survey of aphids and aphid parasitoids in cereal fields in Denmark, and the parasitoids' role in biological control.* Journal of Applied Entomology, 2002. **126**(2-3): p. 101-107.
272. Rossi Stacconi, M.V., et al., *Augmentative releases of Trichopria drosophilae for the suppression of early season Drosophila suzukii populations.* BioControl, 2019.
273. Bowers, C., et al., *Cover crops improve early season natural enemy recruitment and pest management in cotton production.* Biological Control, 2020. **141**: p. 104149.
274. Brewer, M.J., F.B. Peairs, and N.C. Elliott, *Invasive cereal aphids of North America: ecology and pest management.* Annual review of entomology, 2019. **64**: p. 73-93.
275. Figueroa, C.C., et al., *Biological and genetic features of introduced aphid populations in agroecosystems.* Current opinion in insect science, 2018. **26**: p. 63-68.