insects

MDPI

*Article*

# *Aphelinus nigritus* Howard (Hymenoptera: Aphelinidae) Preference for Sorghum Aphid, *Melanaphis sorghi* (Theobald, 1904) (Hemiptera: Aphididae), Honeydew Is Stronger in Johnson Grass, *Sorghum halepense*, Than in Grain Sorghum, *Sorghum bicolor*

**Crys Wright, Anjel M. Helms, Julio S. Bernal, John M. Grunseich and Raul F. Medina ***

Department of Entomology, Texas A&M University, TAMU 2475, College Station, TX 77843-2475, USA
* Correspondence: raul.medina@ag.tamu.edu; Tel.: +1-(979)-845-4775

**Simple Summary:** Like many aphids, the sorghum aphid (SA) *Melanaphis sorghi* produces honeydew, a waste product that can increase parasitoid retention and elicit foraging behaviors on plants. We determined the potential of SA honeydew to retain a common aphid parasitoid, *Aphelinus nigritus*, to assess the practicality of SA biological control on grain sorghum, *Sorghum bicolor*. Since SAs also feed on Johnson grass, *Sorghum halepense*, an alternative plant host, we characterized the composition of honeydew from aphids feeding on either grain sorghum or Johnson grass and evaluated *A. nigritus* preference for honeydew produced on either plant species. Our study found that *A. nigritus* often remained on honeydew produced by SAs feeding on both grain sorghum and Johnson grass. However, despite sharing similar sugar, amino acid, and organic acid profiles, honeydew produced on Johnson grass was preferred by *A. nigritus* over honeydew produced on grain sorghum. Our results suggest that SA honeydew could facilitate *A. nigritus* parasitoid retention on Johnson grass to lower SA populations before the grain sorghum growing season.

**Abstract:** How aphid parasitoids of recent invasive species interact with their hosts can affect the feasibility of biological control. In this study, we focus on a recent invasive pest of US grain sorghum, *Sorghum bicolor*, the sorghum aphid (SA), *Melanaphis sorghi*. Understanding this pest's ecology in the grain sorghum agroecosystem is critical to develop effective control strategies. As parasitoids often use aphid honeydew as a sugar resource, and honeydew is known to mediate parasitoid–aphid interactions, we investigated the ability of SA honeydew to retain the parasitoid *Aphelinus nigritus*. Since SAs in the US have multiple plant hosts, and host–plant diet can modulate parasitoid retention (a major component in host foraging), we measured SA honeydew sugar, organic acid, and amino acid profiles, then assessed via retention time *A. nigritus* preference for honeydew produced on grain sorghum or Johnson grass, *Sorghum halepense*. Compared to a water control, *A. nigritus* spent more time on SA honeydew produced on either host plant. Despite similar honeydew profiles from both plant species, *A. nigritus* preferred honeydew produced on Johnson grass. Our results suggest the potential for SA honeydew to facilitate augmentation strategies aimed at maintaining *A. nigritus* on Johnson grass to suppress SAs before grain sorghum is planted.

**Keywords:** contact kairomones; honeydew; parasitoids; chemical ecology; plant-insect interactions

check for updates

**Citation:** Wright, C.; Helms, A.M.; Bernal, J.S.; Grunseich, J.M.; Medina, R.F. *Aphelinus nigritus* Howard (Hymenoptera: Aphelinidae) Preference for Sorghum Aphid, *Melanaphis sorghi* (Theobald, 1904) (Hemiptera: Aphididae), Honeydew Is Stronger in Johnson Grass, *Sorghum halepense*, Than in Grain Sorghum, *Sorghum bicolor*. *Insects* **2023**, *14*, 10. https://doi.org/10.3390/insects14010010

Academic Editor: Denis J. Wright

Received: 10 October 2022
Revised: 3 November 2022
Accepted: 17 December 2022
Published: 22 December 2022

**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

## 1. Introduction

Parasitoids locate their hosts using a variety of visual, contact, and olfactory cues. In the context of tri-trophic interactions, parasitoid success in finding their hosts is largely influenced by the herbivore's host plant [1–3]. That is because plants contain physical [4] and chemical characteristics [5] that can attract or repel foraging parasitoids [6]. Host plants

can alter parasitoid searching strategy, especially in cases where the same insect host feeds on multiple host plants. Polyphagy in insect hosts can lead to differential parasitoid attack when insect hosts feed on different host plants [6–9]. Among insect hosts, aphids, whose limited mobility make them highly susceptible to parasitism [10–12], produce honeydew, a sugar-rich waste product that can nutritionally supplement parasitoids [13,14]. Since many aphids feed on multiple host plants, plant-mediated differences in honeydew may affect parasitoid retention on this sugar source, as is seen in *Encarsia formosa* parasitoids [15]. Overall, the potential for honeydew-mediated retention of parasitoids, a large component in host-searching and parasitism behaviors, is understudied and warrants attention.

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 [16–18]. Comparatively, fewer studies have assessed the suitability of honeydew, a sugar-rich waste product of aphids and other hemipterans, as a food alternative. Honeydew, which comprises carbohydrates, amino acids, and organic acids [19–23], serves as a food source for some parasitoids [24–28], and predators [27], along with other organisms like ant mutualists [29], inhabiting low-nectar environments. Honeydew utilization is especially common in parasitoids of cereal aphids, even in the presence of nectar sources [30], likely because parasitoids reduce energy-use costs by remaining within a host patch when consuming honeydew instead of actively searching for flowers [27,30].

In addition to a nutritional source, honeydew is considered a kairomone, or a semiochemical host by-product that benefits another species. Aphid by-products, such as honeydew, along with glandular [31] and cornicle secretions [32], are key determinants of parasitoid retention behaviors [33] that increase the efficiency of host location and can improve the likelihood of parasitism [13,14,34]. As a contact kairomone, honeydew 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 [35]. As honeydew can retain parasitoids within infested plants and provide an important food resource, the role of honeydew in parasitoid-mediated aphid suppression merits investigation.

The sorghum aphid (SA), *Melanaphis sorghi*, originally reported as the sugarcane aphid, *Melanaphis sacchari* (Zehntner, 1897), is a cereal pest of economic concern in Texas and has become a serious pest of grain sorghum, *Sorghum bicolor* [36]. Although some studies have documented a combination of management methods and production practices to manage this pest in grain sorghum [37–39], very little is known about the role of natural enemies of SA [37]. 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 feed on host hemolymph to complete egg maturation (Hopper, University of Delaware, pers. communication). When feeding, SAs produce copious amounts of honeydew on grain sorghum, which may provide a potential food resource to this parasitoid. Since parasitoids can also use honeydew as a contact kairomone, SA honeydew might lead to *A. nigritus* retention and facilitate parasitism. Overall, understanding the degree of *A. nigritus* retention on SA honeydew will increase knowledge of the dynamics facilitating parasitism in this parasitoid–host system.

Whereas most honeydews contain a range of sugars, amino acids, and organic acids [26,40–42], the composition and concentration of these macronutrients vary among hemipterans, host plant species, and over time [24,43]. In the case of SA, honeydew varies among cultivars of sugarcane, *Saccharum* spp. [44]. Honeydew variation is important because it can influence 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 [26]. In the case of oligophagous or polyphagous insect hosts, parasitoids may prefer honeydew produced by the same aphid species feeding on different plants [45–47]. Honeydew quantity can also influence parasitoid foraging [25], likely because it reflects the population densities of aphid hosts [30]. 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 more in one plant over another, resulting in skewed parasitism rates.

SA also feeds on the perennial weed Johnson grass, *Sorghum halepense* [48], an uncultivated weed abundant across the southern US [49] that frequently occurs in proximity to grain sorghum. Notably, rates of SA parasitism are reportedly greater on grain sorghum versus Johnson grass (B. Elkins, unpublished data). As host-plant identity largely determines honeydew composition [43], and differences in honeydew may affect parasitoid preference, plant-derived differences in SA honeydew quality and/or quantity could facilitate differences in parasitoid retention and lead to differences in parasitism rates. A better understanding of variations in SA honeydew across host plant species and over time may help determine how parasitoids and other natural enemies modulate their selection of hosts.

In this study, sugar, amino acid, and organic acid compositions and concentrations of honeydew from aphids feeding on grain sorghum or Johnson grass were compared. Subsequently, *A. nigritus* retention on honeydew from aphids feeding on these two host plants was assessed at different collection timepoints (i.e., after honeydew accumulated for 24, 72, or 120 h). As shown in other studies, we expected to detect differences in SA honeydew composition between host plants and over time. Considering the trend of less parasitism in Johnson grass, we also predicted a strong *A. nigritus* preference for SA honeydew produced on grain sorghum. Finally, and since parasitoid foraging generally increases with larger quantities of honeydew [30,50], we further predicted a stronger *A. nigritus* response to SA honeydew produced on grain sorghum when collected over a period of 120 versus 24 h.

## 2. Materials and Methods

### *2.1. Establishing Main Aphid Colonies*

SAs were reared on either DEKALB® DKS 4420 (Bayer, St. Louis, MO, USA), 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). Hence, 3–5 sorghum seeds were planted in 3.8 cm diameter × 21 cm high planting tubes (Amazon, Seattle, WA, USA) containing Sun Gro® Metro-Mix® 360 (Sun Gro® Horticulture, Agawam, MA, USA). 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 × 30 × 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, USA) 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.

### *2.2. Establishing Clonal Aphid Colonies*

Aphid clonal colony cages were constructed from one-liter plastic bottles. A three-mm 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.

### 2.3. 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. *A. 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, USA). 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 h were used in experiments. Once emerged, females were transferred to separate 1.5 mL centrifuge tubes (VWR International, Radnor, PA, USA) containing two µL of autoclaved water (smeared on the sides of the tube to allow hydration while preventing drowning) and a 0–72 h-old male. Females and males were observed until mating occurred.

### 2.4. Honeydew Collection

Ten 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 three-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 three timepoints: 24, 72, or 120 h. After freeze-drying for 24 h, the honeydew disks were weighed, then diluted in 15 µL High Pressure Liquid Chromatography (HPLC)-grade water (Sigma-Aldrich, St. Louis, MO, USA) filtered through spin columns (Thermo Fisher Scientific, Waltham, MA, USA) and stored in 1.5 mL centrifuge tubes at −20 °C until use. Twenty replicates (clip cages) per plant species and timepoint were used for HPLC analysis, while 20 additional replicates were used in bioassays to assess parasitoid preference.

### 2.5. Analysis of Honeydew Composition Using HPLC

Sugar and organic acid composition of SA honeydew was analyzed using an Agilent 1200 HPLC connected to a 1260 Infinity II refractive index detector (Agilent Technologies, Santa Clara, CA, USA). Samples were thawed and sonicated for two minutes, then spun down in a centrifuge at 13,500 rpm. Samples were then transferred to two ml screw top vials containing 150 µL glass inserts (Agilent Technologies, Santa Clara, CA, USA) and run on the Agilent 1200 binary LC gradient system using the Hi-Plex Ca (Duo) 7.7 × 50 mm column and 8 µL guard column (Agilent Technologies, Santa Clara, CA, USA). The column was eluted with 100% HPLC water at a flow rate of 4.0 mL/min at 80 °C [51]. The amino acid composition of SA honeydew was analyzed using an Agilent 1200 HPLC connected to a diode array detector (Agilent Technologies, Santa Clara, CA, USA). Samples were separated using the AdvanceBio AAA 2.7 µm 4.6 × 100 mm column with a 2.7 µm guard column (Agilent Technologies, Santa Clara, CA, USA). Two eluent mixtures (A:10 mM Na2HPO4–10 mM Na2B4O7 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 1.2 mL/min at 40 °C. Prior to injection, amino acids were derivatized with o-phthalaldehyde (OPA), 9-fluorenylmethyloxycarbonyl (FMOC), a borate buffer, and injection diluent containing 100 mL of eluent mixture A and 0.4 mL concentrated H3PO4 (Agilent Technologies, Santa Clara, CA, USA) to create fluorescent molecules for enhanced detection at the 390 nm DAD wavelength [52]. 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, MO, USA). Quantities for each compound were determined by comparing peak areas to calibration curves.

### 2.6. Statistical Analyses of Honeydew Composition

Statistical analyses were performed using R, Version 4.0.5 (R Core Team, Vienna, Austria). Using the R-package VEGAN [53], honeydew sugar and amino acid content were analyzed by conducting permutational multivariate analysis of variance (PERMANOVA) to quantify differences in blends [54] and non-metric multidimensional scaling ordinations to visualize blend differences at different collection timepoints and between host plants [54]. 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. One-way ANOVAs were used to compare individual sugar, amino acid, and organic acid compounds between host plants within collection timepoints.

### 2.7. Measuring Parasitoid Preference

Parasitoid preference for either honeydew source was measured at two timepoints: 24 and 120 h. Hence, 5 µL of honeydew collected after 24 and 120 h (from each of the 20-grain sorghum and Johnson grass replicate plants mentioned above) were 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 × 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 five minutes before recording behavior for 10 min using the Behavioral Observation Research Interactive Software (BORIS, Turin, PIE, Italy) [55]. Preference was measured as the relative proportion of time spent (relative retention) on each treatment (=total time on one treatment leaf/the total time spent on both treatment leaves). To test parasitoid retention on 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.

### 2.8. Statistical Analyses of Parasitoid Preference

Statistical analyses were performed using JMP®, Version 15.2 (SAS Institute Inc., Cary, NC, USA). 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 h) were compared to assess the strength of retention on 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 effect of two available sources of honeydew (grain sorghum versus Johnson grass) on parasitoid preference, along with collection timepoint and original parasitoid aphid host was measured in a separate ANCOVA. For the first ANCOVA comparison, 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. For the second 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. The data were converted to arcsine square-root values (ASIN (SQRT ×)) × 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 retention on one honeydew source over another, between honeydew versus water experiments. Honeydew concentration (dry weight in µg/15 µL HPLC-grade water) was used as a covariate to account for differences in concentration between 24- and 120-h and grain sorghum and Johnson grass honeydew samples. One-way ANOVAs 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).

## 3. Results

### *3.1. SAs Fed on Grain Sorghum and Johnson Grass Excrete Varied Concentrations of Honeydew*

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 1). Overall, sugar concentration was greater in Johnson grass samples ($F_{2,104}$ = 6.0142, $p$ = 0.0159) (Figure 1A). Total sugar concentrations increased over time, although sugar concentration did not vary between the 72 and 120-h timepoints (Figure 1B). Despite this, there was a trend towards greater concentrations of sugar in Johnson grass versus grain sorghum at each timepoint.

**Table 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 × Host Plant | 0.4947 | 2.104 | 0.6112 |

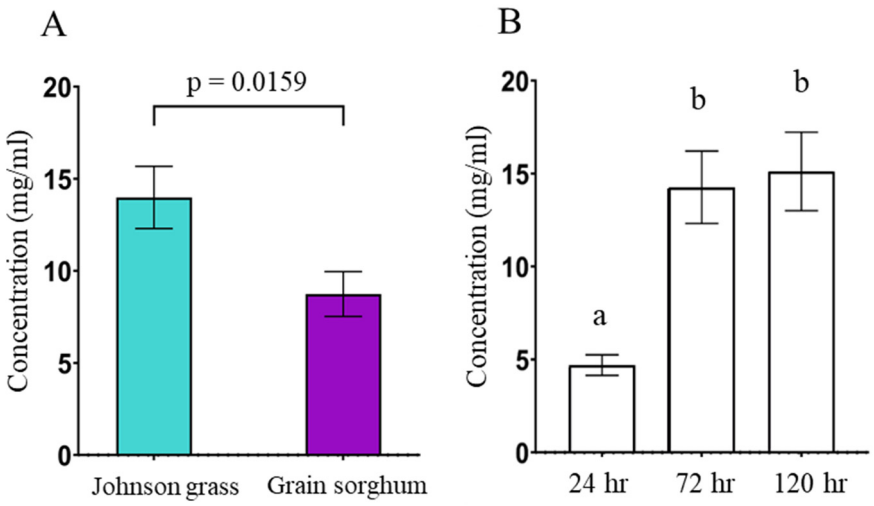



**Figure 1.** (**A**) Overall, sugar concentration is greater in honeydew from Johnson grass aphids (see Table 1). Statistical significance at $p < 0.05$. (**B**) Total sugar concentration of SA honeydew was significantly lower at the 24-h timepoint but no significant difference in sugar concentration was detected between the 72 and 120-hr timepoints. As per Tukey's HSD. Different letters indicate statistical significance across timepoints at $p < 0.05$.

### *3.2. 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 $F_{1,34}$ = 3.0852, $p$ = 0.06) (Figure 2A) and 72 (PERMANOVA $F_{1,34}$ = 1.5388, $p$ = 0.212) hours (Figure 2B). There was a significant difference in profiles between honeydews collected after 120 h (PERMANOVA $F_{1,34}$ = 3.7925, $p$ = 0.01) (Figure 2C). The sugars detected in grain sorghum and Johnson grass honeydew samples are shown in Table 2. Fructose was marginally more abundant in Johnson grass honeydew than in grain sorghum honeydew after 72 h ($F_{1,34}$ = 4.215, $p$ = 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 ($F_{1,34}$ = 10.71, $p$ = 0.0025) and 120-h ($F_{1,34}$ = 5.325, $p$ = 0.0272) collection timepoints (Table 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 h ($F_{1,34}$ = 6.04, $p$ = 0.0198) and Tyrosine after 24 h ($F_{1,34}$ = 9.401, $p$ = 0.0041) (Table S1).

### *3.3. Aphelinus Nigritus Retention Behavior Observed on 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 3). 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 3A). Given the choice between Johnson grass honeydew and water, grain sorghum parasitoids preferred Johnson grass honeydew at both 24-h and 120-h timepoints. However, Johnson grass parasitoids preferred Johnson grass honeydew at the 24-h timepoint but made no choice at the 120-h timepoint (Figure 3B).

**Table 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 h** | | **72 h** | | **120 h** | |
|---|---|---|---|---|---|---|
| **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** |

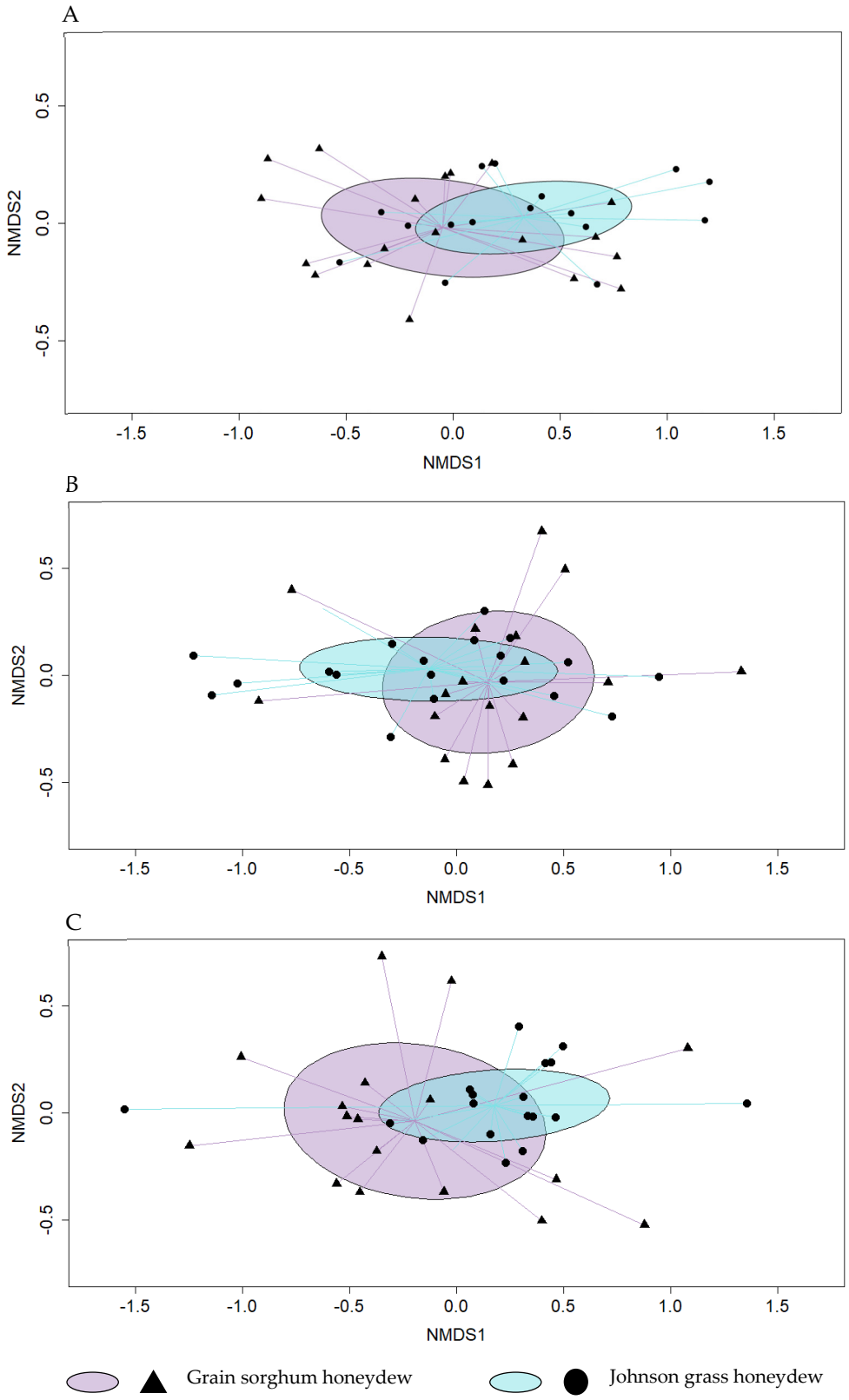



**Figure 2.** Non-metric multidimensional scaling (NMDS) ordination diagram. Sugar and organic acid profiles are similar between grain sorghum and Johnson grass honeydew collected after 24 h (**A**) and 72 h (**B**). Profiles are significantly different between grain sorghum and Johnson grass honeydew collected after 120 h (PERMANOVA $F_{1,34} = 3.7925$, $p = 0.01$) (C). Ellipses represent 95% confidence intervals. Triangle and circle points ordinated closer to one another are more similar.

**Table 3.** Neither Timepoint nor Original Parasitoid Aphid Host Mediated 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 × 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 |

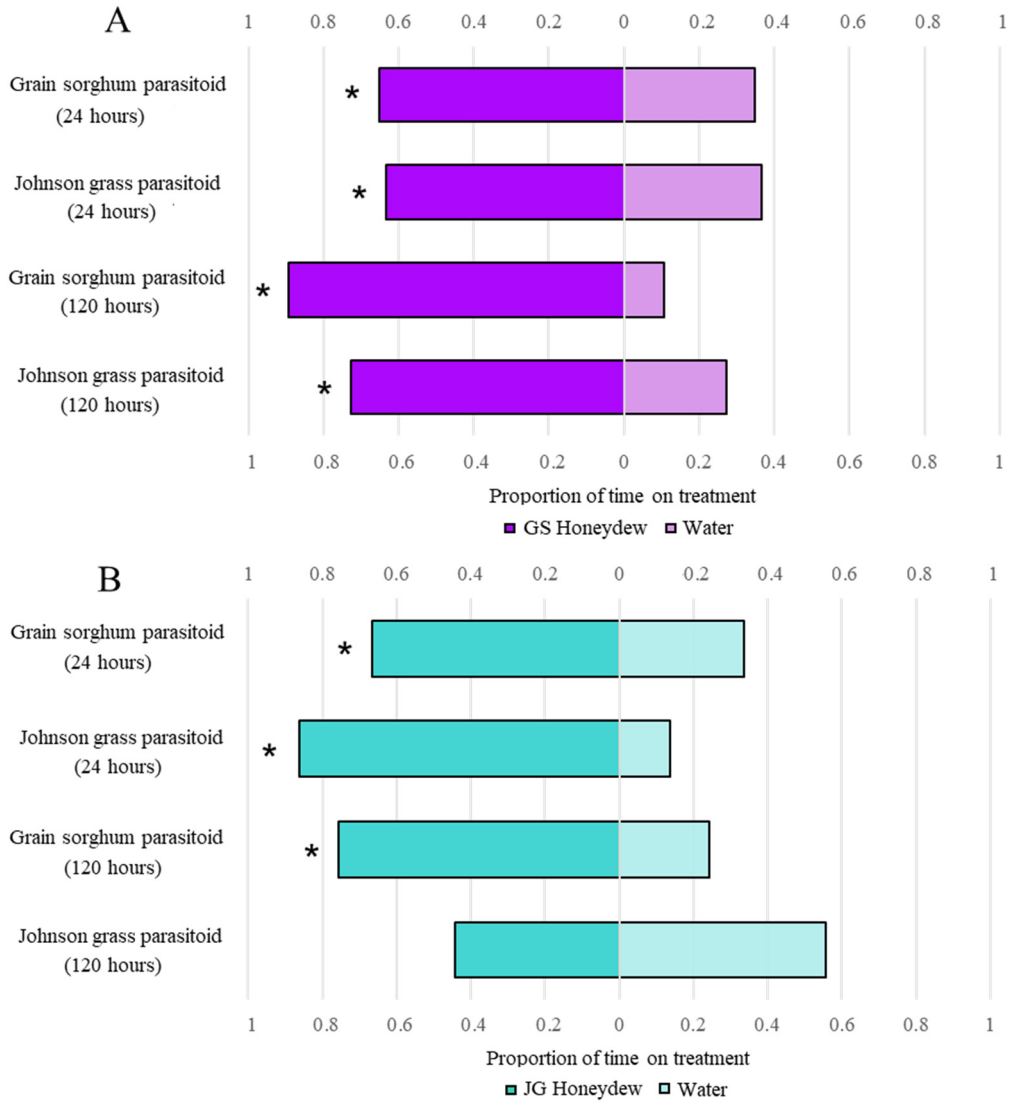



**Figure 3.** (**A**) Grain sorghum and Johnson grass parasitoid retention is greater on grain sorghum honeydew. (**B**) Grain sorghum and Johnson grass parasitoid retention is largely greater on Johnson grass honeydew. As per a one-sample *t*-test, an asterisk indicates statistical significance at $p < 0.05$.

### *3.4. 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 4). At the 24-h timepoint, grain sorghum parasitoids preferred Johnson grass honeydew,

while Johnson grass parasitoids preferred grain sorghum honeydew. At 120 h, both grain sorghum and Johnson grass parasitoids preferred Johnson grass honeydew (Figure 4).

**Table 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 versus Johnson Grass Honeydew.

| Variables | ANCOVA | | |
|---|---|---|---|
| | **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 |

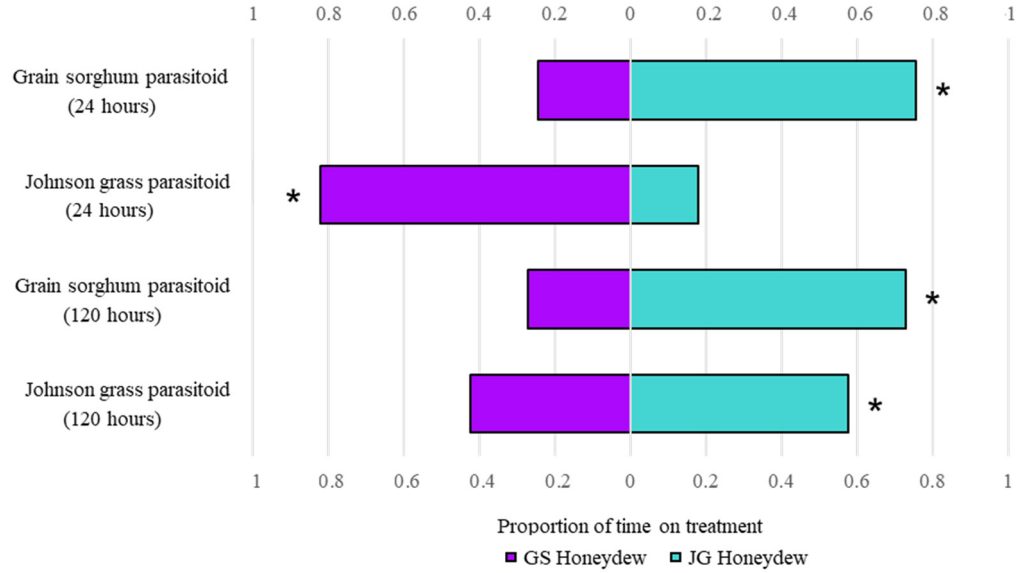



**Figure 4.** Both grain sorghum and Johnson grass parasitoids preferred Johnson grass honeydew, except for Johnson grass parasitoids at the 24-h timepoint (one-sample *t*-test). As per a one-sample *t*-test, an asterisk indicates statistical significance at $p < 0.05$.

## 4. Discussion

*Aphelinus nigritus* preferred SA honeydew produced by SAs feeding on Johnson grass over that produced 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 SAs feeding on Johnson grass or grain sorghum. The sugar and organic acid profiles were mostly similar between honeydews produced by SAs feeding on Johnson grass and grain sorghum, except for honeydews collected after 120 h. Differences at this timepoint appear to stem from a greater accumulation of fumaric acid in Johnson grass honeydew. No study to our knowledge has assessed the role of fumaric acid in parasitoid retention. Consequently, it is unknown as to whether this organic acid affects parasitoid foraging on Johnson grass honeydew. Amino acid profiles were similar between host plants across all timepoints. Approximately 70% of all detected amino acids consisted of aspartic acid, glutamic acid, proline, and serine, all nonessential amino acids for aphids [21,26,56], which obtain essential amino acids from the obligate bacterial endosymbiont *Buchnera aphidicola* [57]. 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 [58,59] and detected in Johnson grass honeydew samples in this study.

We 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 tissues. However, despite studies separately assessing sugar concentration in Johnson grass rhizomes and leaves [60,61] and grain sorghum kernels [62], direct measurements of differences in phloem sugar concentration between these two plant species have not been attempted, to our knowledge. Future comparisons of phloem sugar concentration between plant species might help explain our results. If sugar concentration is greater in grain sorghum plants, a higher sugar concentration in aphid honeydew feeding on Johnson grass could result from compensatory aphid feeding. Aphids can adjust their dietary intake based on nutritional (mainly nitrogen) requirements [63] and nutrient supply [64,65]. It is possible that SAs consumed more Johnson grass phloem, leading to higher honeydew sugar concentration, to meet their nitrogen needs. In addition to measuring phloem sugars, this hypothesis could be validated by measuring phloem amino acid content (a major source of nitrogen) and using electrical penetration graphs to measure SA feeding duration and frequency on both plant species.

Through the honeydew preference bioassays, it was evident that honeydew from either plant species led to *A. nigritus* retention. In the honeydew versus water choice tests, honeydew from aphids fed on the two host plants tested elicited *A. nigritus* arrestment, 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 [15,66,67], suggesting that *A. nigritus* may use honeydew as an SA foraging cue. This is not always common in parasitoids, likely due to high levels of crystallized oligosaccharide sugars that may reduce honeydew palatability [68]. Along with crystallization, high viscosity can also make honeydew sugars difficult to consume [24], which is why parasitoids tend to prefer less viscous nectar resources [69]. 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 are 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 [30]. The decision to consume sugar within host patches likely reduces energy use and host searching costs, creating selective pressure to evolve honeydew consumption instead of relying on nectar and the associated active searching for flowers [70,71].

The original parasitoid aphid host did 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 [67,72,73], which were the three most abundant sugars in SA honeydew from either host plant species. It is important to remember that like most parasitoids, *A. nigritus* would require some source of sugar to maintain enough energy for foraging [74]. Thus, females would likely not reject available honeydew, irrespective of the host plant, as corroborated by our honeydew versus water choice tests, 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 with field observations of higher *A. nigritus* parasitism rates on grain sorghum (B. Elkins, unpublished data) and suggest that *A. nigritus* honeydew preferences may not align with their host oviposition preference. Since the factors affecting field parasitism rates are complex in nature, differing *A. nigritus* parasitism rates may reflect physical instead of chemical plant traits. Early-stage grain sorghum and Johnson grass plants (like the three-week-old plants used in this study) are similar in texture, and in preliminary trials measuring *A. nigritus* preference for water present on grain sorghum and Johnson grass leaves, we found no inherent bias towards leaf type (data not shown). However, compared to grain sorghum, mature Johnson grass has more bicellular trichomes that can lead to microroughness on the leaves [75]. Leaf surface can influence parasitism rates of the same host feeding on multiple plants. For example, Mulatu et al. [76] 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 [77]. Moreover, our study used naïve parasitoids. As a result, we did not consider parasitoid learning, a behavior that can influence host preference [78] and may contribute to observed *A. nigritus* parasitism rates.

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 24 h. This result, along with the observations of no preference in Johnson grass parasitoids between Johnson grass honeydew collected after 120 h and water are perplexing. Since honeydew is a suitable medium for microbial growth [23,79,80], 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 reveal evidence of any correlation between microbial composition and parasitoid preferences for honeydew as measured in this study. We hypothesize that the variation in parasitoid honeydew preference may not correlate with the presence of microbial organisms themselves, but rather with the differential presence of microbial metabolites or microbial volatile organic compounds (MVOCs) in honeydew from different host-plant species, which are both shown to mediate natural enemy activity. For example, some metabolites of the bacterium *Bacillus thuringiensis* are known to repel and deter oviposition of *Eretmocerus eremicus* parasitoids on whiteflies [81]. With respect to volatiles, Fand et al. [82] isolated VOC-producing bacteria from grapevine mealybug honeydew that was highly attractive to the endoparasitoid *Anagyrus dactylopii*. Similarly, Leroy et al. [23] isolated microorganisms' volatiles from pea aphid honeydew that attracted and enhanced predation by hoverflies. Based on our results, the microbial metabolites or MVOCs present in SA honeydew fed on different diets should be assessed and their role in mediating *A. nigritus* honeydew preferences tested.

Despite an apparent host preference for SAs feeding on grain sorghum, *A. nigritus* retention on Johnson grass SA honeydew has positive implications for future honeydew-related pest management, as it suggests that Johnson grass honeydew can maintain parasitoids on SA-infested plants. In the long term, this might lead to the 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 retention can lead to SA suppression is another relevant question that should inform any future assessments of this parasitoid's potential as a biocontrol agent of SA.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects14010010/s1, Table S1: Proportional Composition of Amino Acids in Grain Sorghum and Johnson Grass Sorghum aphid Honeydew Over Time.

**Author Contributions:** Conceptualization, C.W. and R.F.M.; methodology, C.W., R.F.M., A.M.H., J.S.B. and J.M.G.; data curation, C.W.; resources, A.M.H.; investigation, C.W.; formal analysis, C.W., A.M.H., J.S.B. and J.M.G.; software, J.M.G.; visualization, C.W. and J.M.G.; writing—original draft preparation, C.W.; writing—review and editing, C.W., R.F.M., A.M.H., J.S.B. and J.M.G.; supervision, R.F.M.; project administration, C.W.; funding acquisition, R.F.M., C.W. and A.M.H. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Southern IPM Center, grant number 2018-3200-25.

**Data Availability Statement:** Data regarding this study can be found at the Texas Data Repository (https://doi.org/10.18738/T8/DYI6PA, accessed on 20 December 2022).

**Acknowledgments:** We would like to acknowledge Keyan Zhu-Salzman, Ronnie Schnell and Suhas Vyavhare for their review of earlier versions of this manuscript. This work was made possible in part by the Southern IPM Center under grant number 2018-3200-25.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References


1. Takabayashi, J.; Sato, Y.; Horikoshi, M.; Yamaoka, R.; Yano, S.; Ohsaki, N.; Dicke, M. Plant effects on parasitoid foraging: Differences between two tritrophic systems. *Biol. Control* **1998**, *11*, 97–103. [CrossRef]
2. Feng, Y.; Wratten, S.; Sandhu, H.; Keller, M. Host plants affect the foraging success of two parasitoids that attack light brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae). *PLoS ONE* **2015**, *10*, e0124773. [CrossRef] [PubMed]
3. Wäschke, N.; Meiners, T.; Rostás, M. Foraging strategies of parasitoids in complex chemical environments. In *Chemical Ecology of Insect Parasitoids*; John Wiley & Sons: Hoboken, NJ, USA, 2013; pp. 37–63.
4. Lovinger, A.; Liewehr, D.; Lamp, W.O. Glandular trichomes on alfalfa impede searching behavior of the potato leafhopper parasitoid. *Biol. Control* **2000**, *18*, 187–192. [CrossRef]
5. Ye, M.; Veyrat, N.; Xu, H.; Hu, L.; Turlings, T.C.; Erb, M. An herbivore-induced plant volatile reduces parasitoid attraction by changing the smell of caterpillars. *Sci. Adv.* **2018**, *4*, eaar4767. [CrossRef]
6. Liu, S.-S.; Jiang, L.-H. Differential parasitism of *Plutella xylostella* (Lepidoptera: Plutellidae) larvae by the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) on two host plant species. *Bull. Entomol. Res.* **2003**, *93*, 65. [CrossRef]
7. Lill, J.; Marquis, R.; Ricklefs, R. Host plants influence parasitism of forest caterpillars. *Nature* **2002**, *417*, 170–173. [CrossRef]
8. Heard, S.B.; Stireman, J.O.; Nason, J.D.; Cox, G.H.; Kolacz, C.R.; Brown, J.M. 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*, 421. [CrossRef]
9. Elkhouly, A.; Al Hireereeq, E.A.; ALfaqi, A.B.; Shafsha, H.A. 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 J. Agric. Hortic. Res.* **2018**, *1*, 1–7. [CrossRef]
10. Vosteen, I.; Gershenzon, J.; Kunert, G. Enemy-free space promotes maintenance of host races in an aphid species. *Oecologia* **2016**, *181*, 659–672. [CrossRef]
11. Holt, R.D.; Lawton, J.H. Apparent competition and enemy-free space in insect host-parasitoid communities. *Am. Nat.* **1993**, *142*, 623–645. [CrossRef]
12. Gross, P. Insect behavioral and morphological defenses against parasitoids. *Annu. Rev. Entomol.* **1993**, *38*, 251–273. [CrossRef]
13. Budenberg, W. Honeydew as a contact kairomone for aphid parasitoids. *Entomol. Exp. Et Appl.* **1990**, *55*, 139–148. [CrossRef]
14. Lou, Y.G.; Cheng, J.A. Host-recognition kairomone from Sogatella furcifera for the parasitoid Anagrus nilaparvatae. *Entomol. Exp. Et Appl.* **2001**, *101*, 59–67. [CrossRef]
15. Romeis, J.; Zebitz, C. Searching behaviour of Encarsia formosa as mediated by colour and honeydew. *Entomol. Exp. Et Appl.* **1997**, *82*, 299–309. [CrossRef]
16. Lee, J.C.; Heimpel, G.E.; Leibee, G.L. Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. *Entomol. Exp. Et Appl.* **2004**, *111*, 189–199. [CrossRef]
17. Patt, J.M.; Hamilton, G.C.; Lashomb, J.H. Foraging success of parasitoid wasps on flowers: Interplay of insect morphology, floral architecture and searching behavior. *Entomol. Exp. Et Appl.* **1997**, *83*, 21–30. [CrossRef]
18. Lee, J.C.; Andow, D.A.; Heimpel, G.E. Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. *Ecol. Entomol.* **2006**, *31*, 470–480. [CrossRef]
19. Woodring, J.; Wiedemann, R.; Fischer, M.; Hoffmann, K.; Völkl, W. 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*). *Physiol. Entomol.* **2004**, *29*, 311–319. [CrossRef]
20. Byrne, D.N.; Miller, W.B. Carbohydrate and amino acid composition of phloem sap and honeydew produced by Bemisia tabaci. *J. Insect Physiol.* **1990**, *36*, 433–439. [CrossRef]
21. Yao, I.; Akimoto, S.I. Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid Tuberculatus quercicola. *Ecol. Entomol.* **2002**, *27*, 745–752. [CrossRef]
22. Blüthgen, N.; Gottsberger, G.; Fiedler, K. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. *Austral Ecol.* **2004**, *29*, 418–429. [CrossRef]
23. Leroy, P.D.; Sabri, A.; Heuskin, S.; Thonart, P.; Lognay, G.; Verheggen, F.J.; Francis, F.; Brostaux, Y.; Felton, G.W.; Haubruge, E. Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. *Nat. Commun.* **2011**, *2*, 348. [CrossRef] [PubMed]
24. Faria, C.A.; Wäckers, F.L.; Turlings, T.C. The nutritional value of aphid honeydew for non-aphid parasitoids. *Basic Appl. Ecol.* **2008**, *9*, 286–297. [CrossRef]
25. Tena, A.; Wackers, F.L.; Heimpel, G.E.; Urbaneja, A.; Pekas, A. Parasitoid nutritional ecology in a community context: The importance of honeydew and implications for biological control. *Curr Opin Insect Sci* **2016**, *14*, 100–104. [CrossRef]
26. Hogervorst, P.A.; Wäckers, F.L.; Romeis, J. Effects of honeydew sugar composition on the longevity of Aphidius ervi. *Entomol. Exp. Et Appl.* **2007**, *122*, 223–232. [CrossRef]
27. Wäckers, F.L.; Van Rijn, P.C.; Heimpel, G.E. Honeydew as a food source for natural enemies: Making the best of a bad meal? *Biol. Control* **2008**, *45*, 176–184. [CrossRef]
28. Casas, J.; Driessen, G.; Mandon, N.; Wielaard, S.; Desouhant, E.; Van Alphen, J.; Lapchin, L.; Rivero, A.; Christides, J.P.; Bernstein, C. Energy dynamics in a parasitoid foraging in the wild. *J. Anim. Ecol.* **2003**, *72*, 691–697. [CrossRef]

29. Shik, J.Z.; Kay, A.D.; Silverman, J. Aphid honeydew provides a nutritionally balanced resource for incipient Argentine ant mutualists. *Anim. Behav.* **2014**, *95*, 33–39. [CrossRef]
30. Vollhardt, I.M.; Bianchi, F.J.; Wäckers, F.L.; Thies, C.; Tscharntke, T. Spatial distribution of flower vs. honeydew resources in cereal fields may affect aphid parasitism. *Biol. Control* **2010**, *53*, 204–213. [CrossRef]
31. Strand, M.R.; Vinson, S.B. Behavioral response of the parasitoid Cardiochiles nigriceps to a kairomone. *Entomol. Exp. Et Appl.* **1982**, *31*, 308–315. [CrossRef]
32. Battaglia, D.; Pennacchio, F.; Marincola, G.; Tranfaglia, A. Cornicle secretion of *Acyrthosiphon pisum* (Homoptera: Aphididae) as a contact kairomone for the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Eur. J. Entomol.* **1993**, *90*, 423–428.
33. Afsheen, S.; Wang, X.; Li, R.; Zhu, C.S.; Lou, Y.G. Differential attraction of parasitoids in relation to specificity of kairomones from herbivores and their by-products. *Insect Sci.* **2008**, *15*, 381–397. [CrossRef]
34. Brown, R.L.; El-Sayed, A.M.; Unelius, C.R.; Beggs, J.R.; Suckling, D.M. Invasive Vespula wasps utilize kairomones to exploit honeydew produced by sooty scale insects, Ultracoelostoma. *J. Chem. Ecol.* **2015**, *41*, 1018–1027. [CrossRef] [PubMed]
35. Mehrnejad, M.R.; Copland, M.J. Behavioral responses of the parasitoid *Psyllaephagus pistaciae* (Hymenoptera: Encyrtidae) to host plant volatiles and honeydew. *Entomol. Sci.* **2006**, *9*, 31–37. [CrossRef]
36. Nibouche, S.; Costet, L.; Medina, R.F.; Holt, J.R.; Sadeyen, J.; Zoogones, A.-S.; Brown, P.; Blackman, R.L. Morphometric and molecular discrimination of the sugarcane aphid, *Melanaphis sacchari*, (Zehntner, 1897) and the sorghum aphid *Melanaphis sorghi* (Theobald, 1904). *PLoS ONE* **2021**, *16*, e0241881. [CrossRef] [PubMed]
37. Uyi, O.; Lahiri, S.; Ni, X.; Buntin, D.; Jacobson, A.; Reay-Jones, F.P.; Punnuri, S.; Huseth, A.S.; Toews, M.D. Host plant resistance, foliar insecticide application and natural enemies play a role in the management of *Melanaphis sorghi* (Hemiptera: Aphididae) in grain sorghum. *Front. Plant Sci.* **2022**, *13*, 1006225. [CrossRef]
38. Lahiri, S.; Ni, X.; Buntin, G.D.; Punnuri, S.; Jacobson, A.; Reay-Jones, F.P.; Toews, M.D. Combining host plant resistance and foliar insecticide application to manage *Melanaphis sacchari* (Hemiptera: Aphididae) in grain sorghum. *Int. J. Pest Manag.* **2021**, *67*, 10–19. [CrossRef]
39. Seiter, N.J.; Miskelley, A.D.; Lorenz, G.M.; Joshi, N.K.; Studebaker, G.E.; Kelley, J.P. Impact of planting date on *Melanaphis sacchari* (Hemiptera: Aphididae) population dynamics and grain sorghum yield. *J. Econ. Entomol.* **2019**, *112*, 2731–2736. [CrossRef]
40. Völkl, W.; Woodring, J.; Fischer, M.; Lorenz, M.W.; Hoffmann, K.H. Ant-aphid mutualisms: The impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia* **1999**, *118*, 483–491. [CrossRef]
41. Detrain, C.; Verheggen, F.J.; Diez, L.; Wathelet, B.; Haubruge, E. Aphid–ant mutualism: How honeydew sugars influence the behaviour of ant scouts. *Physiol. Entomol.* **2010**, *35*, 168–174. [CrossRef]
42. Zhou, A.; Wu, D.; Liang, G.; Lu, Y.; Xu, Y. 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*, 492–500. [CrossRef]
43. Hendrix, D.L.; Wei, Y.-a.; Leggett, J.E. Homopteran honeydew sugar composition is determined by both the insect and plant species. *Comp. Biochem. Physiol. Part B Comp. Biochem.* **1992**, *101*, 23–27. [CrossRef]
44. Akbar, W.; Showler, A.; Reagan, T.; Davis, J.; Beuzelin, J. Feeding by sugarcane aphid, M elanaphis sacchari, on sugarcane cultivars with differential susceptibility and potential mechanism of resistance. *Entomol. Exp. Et Appl.* **2014**, *150*, 32–44. [CrossRef]
45. Henter, H.J.; Via, S. The Potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution* **1995**, *49*, 427–438.
46. Hufbauer, R.A.; Via, S. Evolution of an aphid-parasitoid interaction: Variation in resistance to parasitism among aphid populations specialized on different plants. *Evolution* **1999**, *53*, 1435–1445. [PubMed]
47. Barbosa, P.; Segarra, A.; Gross, P.; Caldas, A.; Ahlstrom, K.; Carlson, R.; Ferguson, D.; Grissell, E.; Hodges, R.; Marsh, P. Differential parasitism of macrolepidopteran herbivores on two deciduous tree species. *Ecology* **2001**, *82*, 698–704. [CrossRef]
48. EDDMapS; Grass, J. Sorghum halepense Distribution. In *Early Detection & Distribution Mapping System*; The University of Georgia-Center for Invasive Species and Ecosystem Health: Athens, GA, USA, 2019.
49. McWhorter, C. Introduction and spread of johnsongrass in the United States. *Weed Sci.* **1971**, *19*, 496–500. [CrossRef]
50. Longley, M.; Jepson, P.C. Effects of honeydew and insecticide residues on the distribution of foraging aphid parasitoids under glasshouse and field conditions. *Entomol. Exp. Et Appl.* **1996**, *81*, 189–198. [CrossRef]
51. Gale, C.C.; Lesne, P.; Wilson, C.; Helms, A.M.; Suh, C.P.; Sword, G.A. Foliar herbivory increases sucrose concentration in bracteal extrafloral nectar of cotton. *PLoS ONE* **2021**, *16*, e0258836. [CrossRef]
52. Steppuhn, A.; Wäckers, F. HPLC sugar analysis reveals the nutritional state and the feeding history of parasitoids. *Funct. Ecol.* **2004**, *18*, 812–819. [CrossRef]
53. Oksanen, J.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; O'hara, R.; Simpson, G.L.; Solymos, P.; Stevens, M.H.H.; Wagner, H. Package 'vegan'. *Community Ecol. Package Version* **2013**, *2*, 1–295.
54. Clavijo Mccormick, A.; Gershenzon, J.; Unsicker, S.B. Little peaks with big effects: Establishing the role of minor plant volatiles in plant–insect interactions. *Plant Cell Environ.* **2014**, *37*, 1836–1844. [CrossRef] [PubMed]
55. Friard, O.P.; Gamba, M. *Behavioral Observation Research Interactive Software (BORIS)*; University of Torino Via dell'Accademia Albertina: Torino, PIE, Italy, 2016.
56. Shaaban, B.; Seeburger, V.; Schroeder, A.; Lohaus, G. 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*, e0228171. [CrossRef] [PubMed]

57. Brinza, L.; Viñuelas, J.; Cottret, L.; Calevro, F.; Rahbé, Y.; Febvay, G.; Duport, G.; Colella, S.; Rabatel, A.; Gautier, C. Systemic analysis of the symbiotic function of Buchnera aphidicola, the primary endosymbiont of the pea aphid Acyrthosiphon pisum. *Comptes Rendus Biol.* **2009**, *332*, 1034–1049. [CrossRef]
58. Mohammed, N.A.; Ahmed, I.A.M.; Babiker, E.E. Nutritional evaluation of sorghum flour (*Sorghum bicolor* L. Moench) during processing of injera. *World Acad. Sci. Eng. Technol.* **2011**, *51*, 72–76.
59. Ajakaiye, C.O. Amino acid composition of sorghum grains as influenced by grain maturity, genotype, and nitrogen fertilization. *J. Agric. Food Chem.* **1984**, *32*, 47–50. [CrossRef]
60. McWhorter, C. Water-soluble carbohydrates in johnsongrass. *Weed Sci.* **1974**, *22*, 159–163. [CrossRef]
61. Horowitz, M. Seasonal development of established johnsongrass. *Weed Sci.* **1972**, *20*, 392–395. [CrossRef]
62. Neucere, N.J.; Sumrell, G. Chemical composition of different varieties of grain sorghum. *J. Agric. Food Chem.* **1980**, *28*, 19–21. [CrossRef]
63. Wang, G.; Zhou, J.-J.; Li, Y.; Gou, Y.; Quandahor, P.; Liu, C. Trehalose and glucose levels regulate feeding behavior of the phloem-feeding insect, the pea aphid Acyrthosiphon pisum Harris. *Sci. Rep.* **2021**, *11*, 15864. [CrossRef]
64. Prosser, W.; Simpson, S.; Douglas, A. How an aphid (*Acyrthosiphon pisum*) symbiosis responds to variation in dietary nitrogen. *J. Insect Physiol.* **1992**, *38*, 301–307. [CrossRef]
65. Simpson, S.J.; Abisgold, J.D.; Douglas, A.E. Response of the pea aphid (*Acyrthosiphon pisum*) to variation in dietary levels of sugar and amino acids: The significance of amino acid quality. *J. Insect Physiol.* **1995**, *41*, 71–75. [CrossRef]
66. Shimron, O.; Hefetz, A.; Gerling, D. Arrestment responses of Eretmocerus species and *Encarsia deserti* (Hymenoptera: Aphelinidae) to Bemisia tabaci honeydew. *J. Insect Behav.* **1992**, *5*, 517–526. [CrossRef]
67. Hirose, Y.; Mitsunaga, T.; Yano, E.; Goto, C. 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. *Appl. Entomol. Zool.* **2009**, *44*, 175–181.
68. 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*, 197–201. [CrossRef]
69. Vollhardt, I.M.; Bianchi, F.J.; Wäckers, F.L.; Thies, C.; Tscharntke, T. Nectar vs. honeydew feeding by aphid parasitoids: Does it pay to have a discriminating palate? *Entomol. Exp. Et Appl.* **2010**, *137*, 1–10. [CrossRef]
70. Lewis, W.; Stapel, J.O.; Cortesero, A.M.; Takasu, K. Understanding how parasitoids balance food and host needs: Importance to biological control. *Biol. Control* **1998**, *11*, 175–183. [CrossRef]
71. Křivan, V.; Sirot, E. Searching for food or hosts: The influence of parasitoids behavior on host–parasitoid dynamics. *Theor. Popul. Biol.* **1997**, *51*, 201–209. [CrossRef]
72. Heimpel, G.; Lee, J.; Wu, Z.; Weiser, L.; Wäckers, F.; Jervis, M. Gut sugar analysis in field-caught parasitoids: Adapting methods originally developed for biting flies. *Int. J. Pest Manag.* **2004**, *50*, 193–198. [CrossRef]
73. Zhang, Y.; Yang, N.; Wang, J.; Wan, F. Effect of six carbohydrate sources on the longevity of a whitefly parasitoid Eretmocerus hayati (Hymenoptera: Aphelinidae). *J. Asia-Pac. Entomol.* **2014**, *17*, 723–728. [CrossRef]
74. Tena, A.; Senft, M.; Desneux, N.; Dregni, J.; Heimpel, G.E. The influence of aphid-produced honeydew on parasitoid fitness and nutritional state: A comparative study. *Basic Appl. Ecol.* **2018**, *29*, 55–68. [CrossRef]
75. McWHORTER, C.G.; Paul, R.N.; Ouzts, J.C. Bicellular trichomes of johnsongrass (*Sorghum halepense*) leaves: Morphology, histochemistry, and function. *Weed Sci.* **1995**, *43*, 201–208. [CrossRef]
76. Mulatu, B.; Applebaum, S.W.; Coll, M. Effect of tomato leaf traits on the potato tuber moth and its predominant larval parasitoid: A mechanism for enemy-free space. *Biol. Control* **2006**, *37*, 231–236. [CrossRef]
77. Keller, M.A. Influence of leaf surfaces on movements by the hymenopterous parasitoid Trichogramma exiguum. *Entomol. Exp. Et Appl.* **1987**, *43*, 55–59. [CrossRef]
78. Giunti, G.; Canale, A.; Messing, R.; Donati, E.; Stefanini, C.; Michaud, J.; Benelli, G. Parasitoid learning: Current knowledge and implications for biological control. *Biol. Control* **2015**, *90*, 208–219. [CrossRef]
79. Fischer, C.Y.; Lognay, G.C.; Detrain, C.; Heil, M.; Grigorescu, A.; Sabri, A.; Thonart, P.; Haubruge, E.; Verheggen, F.J. Bacteria may enhance species association in an ant–aphid mutualistic relationship. *Chemoecology* **2015**, *25*, 223–232. [CrossRef]
80. Peach, D.A.; Gries, R.; Young, N.; Lakes, R.; Galloway, E.; Alamsetti, S.K.; Ko, E.; Ly, A.; Gries, G. Attraction of female *Aedes aegypti* (L.) to aphid honeydew. *Insects* **2019**, *10*, 43. [CrossRef]
81. Sanchéz, E.R.; Ramírez, A.G.; Ramírez, A.R.; Angulo, M.G.; Suárez, J.M.T.; Alejo, J.C. Biological activity of Bacillus thuringiensis culture supernatant on Bemisia tabaci and its parasitoid Eretmocerus eremicus. *Trop. Subtrop. Agroecosyst.* **2019**, *22*. Available online: https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/2717 (accessed on 20 December 2022).
82. Fand, B.B.; Amala, U.; Yadav, D.; Rathi, G.; Mhaske, S.; Upadhyay, A.; Shabeer, T.A.; Kumbhar, D. Bacterial volatiles from mealybug honeydew exhibit kairomonal activity toward solitary endoparasitoid Anagyrus dactylopii. *J. Pest Sci.* **2020**, *93*, 195–206. [CrossRef]


**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.