

IDENTIFICATION OF GRAIN SORGHUM
RESISTANCE TO SUGARCANE APHID AND FIELD
TEST OF RESPONSE BY HYMENOPTERA TO
HONEYDEW

By

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IDENTIFICATION OF GRAIN SORGHUM RESISTANCE TO SUGARCANE
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Abstract:

Grain sorghum, *Sorghum bicolor*, is one of the most important crops worldwide as it can be used for human consumption, biofuel, and livestock feed. In 2013, the sugarcane aphid, *Melanaphis sacchari* (SCA), switched host plants from their primary host, sugarcane, to sorghum, and caused substantial losses in sorghum yield. It overwinters on Johnsongrass, *Sorghum halepense*, and sorghum in south Texas and Mexico and has become a perineal pest. When the aphid feeds, it damages plant tissue stunting the plant, causing chlorosis, and reducing yield. SCA colonies quickly multiply and produce large amounts of sugary excretia called honeydew. Plant tolerance is an important tool for managing pest populations and a series of greenhouse trials were conducted to assess sorghum germplasm for resistance to SCA. This study tested 19 new germplasm against 1 resistant and 3 susceptible checks. The plants were evaluated for plant damage (1-9), chlorophyll content, height, and leaf number. The resistance trials discovered 11 new germplasm, which displayed resistance similar to the known resistant with two performing better than the known check. In the field, many Hymenoptera were observed visiting SCA honeydew. A series of field collections in sorghum and Johnsongrass were made to test if SCA honeydew influenced Hymenoptera diversity and abundance. Pan traps (yellow, blue, and white), and yellow sticky traps were used to collect Hymenoptera. A total of 3,950 individuals were collected with 30 families, 124 morphospecies, and 2,384 individuals from sorghum and 29 families, 121 morphospecies, and 1,566 individuals from Johnsongrass. The presence of aphids increased capture rates of the families: Halictidae, Sphecidae, Brachonidae, Encyrtidae, Mymaridae, Diapriidae, Scelionidae, and Formicidae. The Johnsongrass surveys were disrupted by mowing; however, the data were similar to sorghum trials. The results of these experiments show germplasm to combat the SCA is available and that SCA may have a positive influence on native Hymenoptera species by generating a sugar resource. These findings provide the potential for growers to place borders of susceptible sorghum around resistant cultivars to benefit insect diversity and abundance while maintaining yield.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

With the globalization of trade, organisms have attained the ability to traverse large geographic areas, finding themselves in new regions where they can either survive and increase or succumb to the new conditions or species that they encounter. Trade and travel do not represent the only ways for species distributions to change (Ziska et al., 2010). Severe weather patterns, water, changing global temperatures, and increased levels of carbon dioxide that affect host plant vigor can also contribute to establishment and increases in population (Hellmann, et al. 2008). For example, global climate change enables some species to naturally expand their range further north as the climate continues to warm (Dukes et al., 2009).

Only a small number of invasive species have been studied for their invasive characteristics. Roughly 72 species are reported regularly in the literature, whereas many more species likely require scientific analysis (Kenis et al., 2008). In general, potentially more invasive species exist within native ecosystems, which go undocumented as scientific funding and priorities focus on organisms that cause major damage to economies, food security, and human health. Exotic species impact the economy by adversely affecting agriculture or urban landscapes and affect human health through shortages of food and disease transmission (Pyšek & Richardson, 2010). Exotic species often decrease species richness in new environments that they colonize, alter natural resources, change disturbance regimes, and potentially damage any human enterprise that relies on natural environments (Pyšek & Richardson, 2010).

Invasive species annually caused an average \$97-\$120 billion USD in economic losses between 1906-1991, and Europe regularly sees \$12.5-\$20 billion USD in losses annually (Pyšek & Richardson, 2010). These estimates are rather simplistic because of the different factors used in calculating damage. For example, Yahdi et al. (2015) developed a formula for estimating damage and control costs for the potato leafhopper, *Empoasca fabae* (Harris). While economic losses due to yield reductions can be directly measured, many other indirect effects normally go unreported. These indirect effects usually constitute trophic-level cascade effects, exploitative competition, and indirect mutualism or commensalism (White et al., 2006). These indirect effects could have greater consequences to the health of the affected ecosystem. For example, alien pathogens, parasitoids, and predators introduced for biological control have caused non-target effects and economic losses; however, the vast majority of documented cases benefit the recipient ecosystem (Messing & Wright, 2006). Continued research examining indirect effects, whether beneficial or detrimental, of both invasive species and their potential biological control agents is needed (White et al., 2006).

Occasionally, when an invasive organism arrives in a new area, an endemic species may develop a predatory behavior toward it inhibiting the new species expansion. For example, the ant, *Anoplolepis gracilipes* (Smith), normally dominates new territories; however, an endemic toad species, *Ingerophrynus celebensis* (Günther), has been recorded feeding on and even controlling the ant population within cocoa plantations in Indonesia (Wanger et al., 2011). Similarly, the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), is an invasive aphid species from south Asia discovered in the United States in 1983. Research done examining native *Hippodamia* spp. predator response to this new species showed favorable biological control wherein they reduced the aphid densities consistently by 54% across every year and season (Michels et al. 2001).

History of Sugarcane Aphid in the United States

The sugarcane aphid (SCA), *Melanaphis sacchari* (Zehntner), which is native to East Asia, did not make it to Central and North America until 1977 when it was discovered in Florida sugarcane fields (Mead, 1978). After its initial discovery, it spread to Louisiana where it continues to be a pest of sugarcane. In 2013, SCA was discovered on sorghum varieties in both the United States and in Mexico (Rodríguez-del-Bosque & Terán, 2015; Villanueva, 2014) causing major losses of yield, ranging from 30-100%. The aphid's dispersal in the United States was comparatively more gradual than its range expansion in Mexico. During 2013, SCA was only reported in 38 counties from four states (Bowling et al., 2016). Two years later the range had increased to 400 counties in 17 states (Bowling et al., 2016). Texas sorghum production alone lost \$276 million from 2014-2016 (Zapata et al., 2018). The market value for sorghum already constitutes it as a low-value crop, and losses of this magnitude were devastating to many producers.

Biology and Physiology of Sugarcane Aphid

Sugarcane aphid is a hemipteran that feeds on xylem and phloem sap of its host plants (Singh et al., 2004). When SCA colonizes young plants, feeding damage symptoms consist of purple leaves which results from nutrient deficiencies, seedling discoloration from chlorosis, and tissue necrosis. Stunting of the overall plant height can occur along with a delay in the flower stage. This delay can cause the grain to fill in poorly, negatively affecting the quality and overall yield (Singh et al., 2004). In addition to feeding damage, SCA produces honeydew, which disrupts photosynthesis and can allow sooty mold, a diverse group of Ascomycetes fungi, to establish. The development of sooty mold reduces the photosynthetic capacity of the leaf and causes the plant to become stressed from both aphid feeding and malnutrition (Singh et al., 2004).

Populations of the aphid normally do not build up in agricultural systems until later plant growth stages and are most often associated with dry conditions. The aphid colonies feed on the undersides of leaves (Singh et al., 2004) and during intense levels of infestations, aphids can number more than 30,000 individuals per leaf.

Sugarcane aphid reproduces rapidly via parthenogenesis, decreasing their population doubling time (Singh et al., 2004). When conditions deteriorate, SCA can also produce alate parthenogenetic females, which can disperse into new fields. Other aphid species such as *Rhopalosiphum padi* (L.) and *Diuraphis noxia* (Mordvilko) undergo rapid population increases through parthenogenesis, and aphid clustering has been documented to increase development rates and reproductive output (Qureshi & Michaud, 2005). Therefore, SCA has the potential for rapid population growth from a few established females.

Temperature plays an important role in aphid development. Sugarcane aphid developmental thresholds have only recently been examined. de Souza et al. (2019) determined that 15°C benefitted SCA longevity while temperatures that were 10°C or below, and 30°C and higher, prevented reproduction. The authors calculated a lower threshold of 9°C and upper threshold of 32°C for SCA survival and reproduction. This shows that the colonial success, aphid development, and potential range are restricted to specific geographic ranges and may indicate movement northward to find favorable conditions. The populations of the SCA in North America may be higher in areas such as Oklahoma and Kansas if more southern areas become too warm (de Souza et al. 2019).

An interesting phenomenon recently documented by Peña-Martínez et al. (2018) is that SCA females periodically abort nymphs. This spontaneous abortion had two forms. The first type is a simple spontaneous abortion which occurred in a single embryo or nymph. Alternatively, a complex spontaneous abortion affected multiple embryos sequentially. The highest rates of

abortion occurred in greenhouses where the least insecticides were used. Peña-Martínez et al. (2018) suggested that the abortions resulted from physiological processes, parasites, or unidentified abiotic factors (such as plant chemicals). Determining the reasons for this observation could be important in future management of SCA.

Host Specificity of the SCA

Forty-three related monocot plant species have been identified as possible hosts for SCA. When these host plants were tested for their compatibility with SCA, Wilson and Kerns (2017) discovered SCA to demonstrate host specificity rather than polyphagy. When tested through free-choice trials containing both resistant and susceptible *Sorghum* spp. along with other grass species, the aphids favored sorghum. Of those forty-three species, many of them can readily be used in the production of biofuels. As economies continue to move toward the use of alternative fuels for energy, production of these plants will increase. This could then allow the SCA to increase its range and impact on agricultural systems. The biofuel grasses Armstrong et al. (2019) identified that are not favored by SCA were napiergrass, giant reed, and switchgrass. The SCA successfully fed and reproduced on all other tested grasses making them potentially susceptible to SCA.

Despite these results, host plant preference by SCA is not as simple as it would first appear. Because SCA reproduces primarily through parthenogenesis, most populations could be assumed to be genetically similar and have the same feeding habits globally. However, genetic analysis has uncovered small differences within SCA genomes (Footitt et al., 2008; Medina et al., 2017). These differences were categorized by geographic origin of the pest colony rather than host plant (Nibouche et al., 2014). In total, six multilocus lineages have been discovered from among 15 tested locations. The first five biotypes were sampled from 2002-2009 and given the names of MLL-A through MLL-E. Biotype MLL-A most likely originates from Africa; MLL-B

from Australia; MLL-C from South America, the Caribbean, Indian Ocean, and East Africa, inhabiting the largest range of any haplotype; MLL-D from the USA; and MLL-E from China (Nibouche et al., 2014). More recent research in the United States has shown that two distinct biotypes, MLL-D and MLL-F exist. These biotypes were separated both genetically and phenotypically (Paudyal, et al 2019). The newest biotype, MLL-F, is associated with the SCA on sorghum discovered in 2013.

After this invasion and rapid spread in North America, the new MLL-F biotype became the dominant haplotype over the previously recorded MLL-D (Nibouche et al., 2018). These MLLs are genetically different, and Nibouche et al. (2018) hypothesize that the new MLL acquired after 2013 occurred as a result of a new colonization from an otherwise unknown superclone. The differences in the SCA genome translate in differentiated behavioral and developmental parameters. For example, Nibouche et al. (2015) conducted genomic and host plant preference studies on SCA individuals collected from various part of the world. They found different biotypes had varying associations with specific plants.

In addition to causing direct yield losses, the SCA is a vector of several known plant pathogens, including Sugarcane Yellow Leaf Virus, Millet Red Leaf Virus, Sugarcane Mosaic Virus, and Barley Yellow Dwarf Virus (Singh et al., 2004). Of these, Barley Yellow Dwarf is the most important because it affects many different cereal crops and can significantly reduce grain yields (Blackman et al., 1990). This disease is caused by a virus which can be transmitted not only by SCA, but also by an additional 24 aphid species belonging to 15 different genera (Blackman et al., 1990). Sugarcane Yellow Leaf Virus can be acquired by SCA from an infected plant (sorghum or sugarcane) roughly 4.5 hours after feeding, and can be transmitted to new plants after two days (Behary Paray et al., 2011; Singh et al., 2004).

Within sugarcane fields in Louisiana, McAllister et al. (2008) found disease incidence to increase as aphids dispersed. The disease spread across the experimental fields slowly through trivial aphid movement rather than alate dispersal. Uncertainty remains about the duration required for a virus to infect an aphid related to a specific event in its feeding behavior. For *R. padi*, Prado and Tjallingii (1994) found that the deposition of saliva into plant sieve elements resulted in infection with the pathogen because the virus is located within the aphid saliva and termed the stage “E1.” More research is needed to determine if the SCA could be a viable vector, and the duration needed for it to be able to transmit the disease. Because the majority of aphids will attempt feeding on a wide range of plants, potentially transmitting pathogens in the process, further vector research is needed.

Pest Status of Sugarcane Aphid

Sugarcane aphid has been a documented pest of sorghum for many decades around the world prior to becoming a pest in North America. This aphid is distributed in Asia, Africa, and North and South America along with islands in the Gulf of Mexico (H. C. Sharma, 1993). It often causes economic damage and integrated pest management programs face challenges because most of the plants attacked by SCA are low-input, low-value crops. Limits to available resistant cultivars has been suggested to be among the greatest challenges (Sharma, 1993) in preventing losses. For example, van den Berg (2013) noted that the most commonly grown sorghum cultivar in South Africa was very susceptible to SCA leading to annual losses

Surveying for SCA

Monitoring for the presence and relative abundance of SCA is critical to determining if economic thresholds will be met, triggering the need for management action. Currently, aphid infestations are primarily discovered through field crop scouting. Gordy et al. (2019) recently established economic thresholds for SCA in sorghum and created a baseline threshold of 40 aphids per leaf. However, this number is dependent on field conditions, crop value, the sorghum cultivar, and costs associated with management. More efficient ways to monitor fields are being explored.

Recently, the use of drones with attached infrared cameras has been suggested as a monitoring tool. These drones are flown over sorghum plots where suspected aphid infections exist. A software program, Normalized Difference Vegetation Index (NDVI), is used to detect changes in plant physiology. The NDVI program registers small differences in the reflected visible and near-infrared spectra and can then use these data to quantify plant stress (Elliott et al., 2015). Stanton et al. (2017) determined the use of drones configured with NDVI systems could most accurately determine plant stress when comparing plant height and the NDVI values when examining an entire field for differential plant height and heat reflection. However, this method only detects severe infestations and does not help with early detection that would improve the likelihood of successful management before damage occurs (Stanton et al., 2017).

Management with Insecticides

The use of insecticides in highly modified, large-scale agricultural fields persists as a regularly incorporated control method in pest management (Brattsten et al., 1986). Having the ability to reduce pest numbers quickly to preserve yield is part of integrated management and agricultural sustainability. When SCA became a pest in sorghum, there were no products

registered for its control. Since the arrival of SCA, insecticide efficacy trials have been carried out to gain knowledge and approval for use.

Initially, neonicotinoids showed promising results for control of SCA (Jones et al., 2016). In 2014, a new insecticide class called butenolides (e.g., flupyradifurone; trade name, Sivanto Prime) was released. Sivanto Prime was marketed to control resistant insects while being order specific, and thus, safe for non-target insects like bumble bees or honey bees (Nauen et al., 2015). Following the release of this insecticide, research was conducted to determine best application practices and persistence in the environment. For three consecutive years, flupyradifurone displayed consistent high lethality in a variety of crops against a number of pests (Studebaker & Jackson, 2017; Zarrabi et al., 2017, 2018). While early studies showed flupyradifurone to be safe for native pollinators, additional research on potential non-lethal effects is still required.

While most modern insecticides are based on synthetic compounds, some have been developed from biological organisms. For example, an entomopathogenic fungus, *Lecanicillium lecani*, has potential for use as a reduced risk insecticide for aphids (Haar et al., 2018). This fungus was discovered on overwintering aphids and may have caused a population crash in at least one location (Haar et al., 2018).

When producers decide to apply pesticides, they need to be aware of potential non-target effects. Specifically, beneficial insects move in and out of fields foraging for food or prey. Some insecticides can alter the behavior of pollinators making them forage in inefficient ways, or have sublethal effects that reduce lifespan (Challa et al., 2019). Fields treated with spinosad were found to decrease honey bee populations and spinosad along with imidacloprid negatively affected pollinators during flowering stages (Challa et al., 2019). Neonicotinoids have recently been shown to display high toxicity or adversely affect motor functions of pollinators and predators (Jacob et al., 2019).

Combining control methods and reducing pesticide use for management of SCA are the goals of integrated pest management (IPM). Alternatives to conventional pesticides include identifying and breeding host plant resistance, encouraging natural enemies, and applying cultural control techniques.

Host Plant Resistance

While insecticides can be used to suppress aphid populations, environmentally and economically sustainable alternatives should be used in comprehensive IPM programs for aphids. Aphid colonization and yield loss can be mitigated through the identification of resistant varieties (Sharma 1993). Resistant varieties are bred, crossed, and/or hybridized with various germplasm in order to display resistant traits while maintaining yield and other desirable characteristics. Resistance is a plant's innate ability to overcome an herbivore's feeding or oviposition activity. Host plant resistance can be categorized as antixenosis (non-preference), antibiosis, and tolerance (Painter, 1958; Smith et al., 2005).

Antixenosis is distinguished by the plant's ability to make it unrecognizable or unfavorable as a host. In contrast, antibiosis involves physical or chemical mechanisms that defend the plant by adversely affecting the herbivore's biology. Such adverse effects include lower fecundity, reduced life span, slower developmental rate, and possibly death. Tolerance differs from the other two types of resistance because a tolerant plant allows the insect pest to develop, reproduce, and feed without hindering the plant's survival and ability to produce an expected yield. Paudyal (2019) examined different host plant resistance types within sorghum toward the SCA, and outlined her discovery of antixenotic, antibiotic, and tolerance resistance. Antixenosis was evaluated by growing germplasm along the edge of pots, and placing the aphids in the middle allowing them to choose which plant they favored. The resulting germplasm, which

expressed antixenotic traits, harbored lower numbers of aphids when compared to the susceptible checks. Of the 23 lines, 5 lines showed evidence of antixenosis. Likewise, antibiosis was examined through no-choice trials wherein aphids were restricted to a single germplasm, recording their variation of biology and physiology while on different germplasm. Plants which expressed antibiotic traits resulted in lower aphid fecundity, longer developmental times, and shorter lifespans. Again, 5 of the 23 lines displayed strong evidence of antibiotic resistance. Lastly, tolerance was also examined by recording the changes in overall plant health when exposed to the SCA. The five germplasm, which displayed lower damage rating, and higher chlorophyll content, were assumed tolerant.

Tolerance has an advantage over other forms of host plant resistance in that it prevents selective pressure from being applied on a pest, reducing the ability for the pest to overcome the plant's resistance through natural selection (Reese et al., 1994). In addition, a tolerant cultivar promotes natural enemy populations because harmful chemicals do not accumulate in the pest and the pest remains a nutrient-rich food source (Reese et al., 1994).

While breeding for plant resistance is beneficial for agroecosystems, crop-to-weed gene flow can occur, resulting in unwanted hybrid plants that contain resistant genes (Arriola & Ellstrand, 1996). These genes may cause weedy species to become resistant to normal treatments like herbicide application or cultural control methods normal treatments. For sorghum agroecosystems, sorghum-Johnson grass interactions are especially important (Arriola & Ellstrand, 1996). This occurs when johnsongrass grows along the edges of sorghum fields. When the sorghum crop flowers, the pollen may be intercepted by the Johnsongrass as both plants are wind pollinated. This creates hybrid plants that may be resistant to pests or herbicides making a weedy, invasive plant more difficult to control.

This unwanted interaction has been further studied at the gene level. The first scientist to discuss plant-pest interactions through gene interaction was H.H. Flor who is credited with developing the gene-for-gene theory (Flor, 1956). This theory describes how specific genes interact either acting as enablers or disablers for colonization to take place. Specifically, Flor proposed that pathogens need a gene that enables them to be virulent, while the plant needs to express susceptible genes at the same time. Plants normally have a dominant resistant gene while the pest or pathogen expresses a recessive gene for virulence (Flor, 1956). This means that plants are inherently resistant to most pathogens while most pests or pathogens are avirulent to most hosts. To be affected, pests have to express their recessive gene while the plant expresses a recessive susceptible gene simultaneously in order for a disease or new host association to occur. Thus, in order for a pest or pathogen to overcome resistance in a plant, products of a single gene must be surpassed (Ayliffe et al., 2008). Wheat stem rust is a highly plastic fungi which has continually overcome resistant cultivars. This creates a situation where continued development of new resistant germplasm needs to be developed. As plant varieties are developed, often recessive traits are selected because of advantages to yield, drought tolerance, growth form, etc. These traits may also unintentionally reduce a variety's resistance to pathogens or pests. Other genetic combinations in varieties of a plant may provide inherent resistance to pathogens or pests.

Testing for Host Plant Resistance

When developing new cultivars from germplasm, using sound experimental practices during discovery is imperative. Researchers usually start by challenging plant seedlings with a disease or pest. If a plant shows favorable characteristics then further testing it by growing it to a seed-producing stage may be beneficial. For example, research often begins with no-choice and free-choice trials using flats that contain both experimental germplasm along with known

resistant and susceptible lines (Limaje et al., 2018). Follow up research can determine the type of resistance a plant expresses. Limaje et al. (2018) identified germplasm with both antibiotic and tolerance resistance to SCA. When determining tolerance, Limaje et al. (2018) looked at plant damage and chlorophyll content in no-choice trials. Tolerant plants displayed low levels of damage, and maintain high levels or even greater levels of chlorophyll within the leaves. In no-choice experiments focused on identifying tolerance, aphid biomass was also important (Limaje et al., 2018, Paudyal et al., 2020). A plant that is tolerant to SCA displayed similar or even higher levels of aphid populations while showing little damage.

Identifying antixenosis as mechanism for resistance is often more challenging. Tests for antixenosis require that insects choose their preferred host plant without being influenced by the experimental procedures (Paudyal et al., 2019). To carry out these tests, aphids are placed in an experimental enclosure with a number of plants available. The aphids choose plants and begin reproducing, allowing researchers to obtain data for the number of aphids on each cultivar. At the end of the trial, aphid numbers on each plant are compared with the number of aphids on the known susceptible variety, which grew in the same chamber. The germplasm with the fewest aphids is described as displaying the most antixenotic resistance (Wilson & Kerns, 2017, Paudyal et al., 2019).

Host Plant Resistance in Sorghum

Shortly after the initial discovery of SCA on sorghum, a resistant cultivar was identified. Initial tests of cultivars resistant to greenbug, *Schizaphis graminum* (Rondani), for resistance against SCA proved to be successful (Armstrong et al., 2015). The hybrid, Tx2783, expressed tolerant resistance against SCA, along with eight other tested germplasms that displayed moderate levels of resistance against this aphid. Following the success of the initial experiments,

Armstrong et al. (2017) discovered two additional resistant cultivars, B11055 and R13219, which exhibited all three forms of host plant resistance. They also discovered six more resistant sorghum lines exhibiting antibiosis. Additional discoveries followed for grain sorghum (Armstrong et al., 2018).

Experiments identifying resistance type are usually composed of life history analyses. Bayoumy et al. (2016) conducted experiments examining the differences between aphid growth on susceptible and resistant cultivars. When compared to greenbug, SCA developed faster on susceptible germplasm, while developmental time increased on cultivars of sorghum exhibiting antibiosis. The reproductive capabilities of SCA decreased when feeding on resistant lines of sorghum. However, SCAs develop faster than greenbug no matter which sorghum cultivar they colonize (Bayoumy et al., 2016). When tested on resistant cultivars, Tx2752 and Tx2783, SCA populations decreased and developmental time increased leading to slower population doubling times (Brewer et al., 2017). In field tests, the resistant sorghum varieties did not experience yield reduction while the susceptible varieties did.

While SCA has been an agricultural pest in the Americas for less than a century, resistant cultivars have been reported from other areas. For example, Japanese researchers identified three highly resistant as well as fifteen moderately resistant cultivars from China. They also identified three highly resistant and one moderately resistant cultivars originating from Japan (Hagio, 1992). In India, ten new cultivars for resistance have been developed. These cultivars were compared to resistant checks and damage from SCA correlated positively with plant height and negatively with yield (Sharma et al., 2014). Lopes-da-Silva et al. (2014) compared development of SCA on sweet sorghum and sugarcane and found the aphid developed faster and had more progeny on the former crop.

Other plants colonized by SCA have been tested for resistance. Some cultivars of sugarcane express resistance toward two species of aphids, SCA and the yellow sugarcane aphid, *Sipha flava* (Akbar et al., 2010). While sugarcane sustained larger numbers of SCA than *S. flava*, the yield was unaffected. Antibiosis can occur on a micronutrient level. In sugarcane, susceptible and resistant phloem content and resulting aphid honeydew excreta were examined for their amino acid content. Susceptible sugarcane contained far more free and non-essential amino acids when compared to resistant cultivars (Akbar et al., 2014). Not only was the prevalence of amino acids greater in susceptible cultivars, but the diversity of amino acids was also higher with 15 in susceptible sugarcane compared to 11 in resistant. The amino acid content of phloem sap was primarily composed of non-essential amino acids but differed after aphid feeding. Aphids that fed on susceptible sugarcane phloem excreted five essential and two non-essential amino acids. In contrast, aphids feeding on the resistant cultivar only produced three essential amino acids. This difference in amino acids in the phloem potentially inhibited sieve element production during the feeding process (Akbar et al., 2014). While studies examining the amino acids produced by aphid feeding are common, little research has been done on honeydew protein content. Sabri et al. (2013) found the honeydew produced by *Aphis fabae* to contain 140 different proteins along with the amino acids which potentially make it a much more valuable resource than initially expected. Honeydew production by *A. fabae* increased when they fed on perennials rather than annuals (Fischer, 2005). Aphids obtain nutrients from their internal symbionts that process the phloem. The relationship between protein contents in excreta and resistant cultivars may shed more light on the effectiveness of resistant plants toward aphids.

Even though plant sap contains relatively low levels of micronutrients, aphids and other sap-feeding insects must acquire ample nutrients in order to develop. The facultative symbiont, *Buchnera*, is transferred vertically from the mother to her offspring, and they are located in the aphid hemocoel within mycetocytes (Douglas, 1998). Growth rates differed among aphids with

and without *Buchnera*; aphids lacking the bacteria developed more slowly, produced fewer progeny, and were much smaller as adults (Douglas, 1998). When *Acyrtosiphon pisum* received an antibiotic treatment, the aphids produced far fewer proteins in the honeydew (Prosser & Douglas, 1991). While the symbionts cannot produce vitamins or sterols, their ability to transform non-essential amino acids acquired from the phloem into essential amino acids results in the success in aphid development and reproduction (Douglas, 1998).

While symbionts like *Buchnera* primarily assist in supplying nutrients for their hosts, secondary symbionts have also been documented to serve defensive purposes. Oliver et al. (2003) examined the effects of symbionts in pea aphid, *Acyrtosiphon pisum*. They analyzed aphid fitness when faced with a parasitoid wasp, *Aphidius ervi* (Haliday). The secondary facultative symbiont was shown to increase aphid survivorship by granting innate defenses against the parasitoids. Specifically, the parasitoid larvae imbedded within aphids with symbionts were unable to develop properly (Oliver et al., 2003). There has not been any evidence to explain the exact mechanisms of this phenomena, but researchers have postulated the mechanisms could derive from either the host or the endosymbiont. Specifically, the presence of this endosymbiont may change the cause the host immune system to work more effectively than without. Alternatively, the endosymbiont may create an unfavorable environments within the host for the larvae to develop, or it may produce a toxic which kills the larvae while the host is immune (Oliver et al., 2003).

Natural Enemies

No matter where SCA spreads, both native and introduced natural enemies are able to locate, attack, and regulate populations of the aphid. Singh et al. (2004) reviewed the literature and found 47 known natural enemies that use SCA as a prey or host. Five orders of insects,

Coleoptera, Hymenoptera, Diptera, Neuroptera, and Hemiptera) were recorded with the vast majority of the listed species exhibiting predatory behaviors. Surveys in the USA examining the various aphidophagous arthropods associated with an abundance of SCA within sorghum had similar findings.

Typically, the most common insects observed within the fields belong to the families Coccinellidae (Coleoptera), Chrysopidae (Neuroptera), Hemerobiidae (Neuroptera), and Syrphidae (Diptera) (Bowling et al., 2016). Four native coccinellids have been shown to prey upon SCA, aiding in efforts to control this invasive species (Colares et al., 2015). Only three hymenopteran families, Aphelinidae, Aphidiidae, and Encyrtidae, were collected from SCA-infested plants (Bowling et al., 2016; Colares et al., 2015).

The predominant species of natural enemies attacking SCA vary with geographic region, but it appears natural enemies regularly find aphids in agricultural fields (Bowling et al, 2016). Even though there is high diversity among natural enemies of SCA, they typically cannot keep pace with reproduction of the aphid. For example, Brewer et al. (2017) found that parasitoid abundance increased with aphid population growth; however, the reproductive capacity of SCA was not affected. Similarly, coccinellid beetles increased with aphid numbers, but the predators did not prevent damage in most cases (Brewer et al., 2017).

Although parasitoids and predators have been recorded feeding on SCA, detailed work for many species feeding on SCA associated with sorghum is still missing. Life table data are important in identifying the best candidate natural enemies. For example, Raney, Coles, Eikenbary, Morrison, and Starks (1971) conducted life history experiments on *Aphelinus asychis* focusing on its host preference, longevity, developmental period, and sex ratio. They tested the parasitoid's preference among three aphid species, the greenbug (*Schizaphis graminum*), corn leaf aphid (*Rhopalosiphum maidis*), and the yellow sugarcane aphid (*Sipha flava*). The parasitoid laid

eggs in all of the species; however, it preferred greenbug. The life cycle took approximately 14.5, 13.5, and 12.3 days to complete at 25°C depending on the age or life stage. The adult lived from 9 days at 32.2°C to 18 days at 23.9°C. The sex ratio became skewed toward female production as the temperature decreased, but was approximately 50/50 during average and warmer temperatures.

Honeydew can influence many organisms both on the macro and micro level. The potential for a complex relationship among aphid honeydew and other taxa is still relatively unexplored. For example, Leroy et al. (2011) found that hoverflies (Diptera: Syrphidae) will orient themselves and oviposit near cultures of bacteria that were growing on aphid honeydew. They hypothesized this is most likely a form of chemotaxis in response to the volatiles given off from the bacteria as they break down sugars and amino acids contained within honeydew.

Parasitoids have also been shown to utilize sugars from sources other than nectar from floral sources. Some parasitoids will use aphid honeydew as an extra food source if nectar is scarce in their environment, while others may solely use it as a kairomone to locate their hosts from the chemical signals the honeydew emits (Wäckers et al., 2008). The usefulness of honeydew as a food source varies by species, but normally wasps more closely tied to aphids for their life cycle benefit most (Wäckers et al., 2008).

Bees have also been shown to be attracted to honeydew resources exuded from hemipterans. While research is sparse on this topic, important observations have been made. Namely, Meiners, et al. (2017), noted that bees were able to locate honeydew resources by olfactory cues or by seeing other bees aggregate in a single area. The authors further suggest that honeydew may provide a necessary resource during flowerless times.

Honeydew can also help protect aphids by encouraging symbiotic relationships with ants. This relationship is regularly noted in the literature among many different species. The aphids

produce honeydew for ants, and the ants provide defensive services for the aphids. This allows ants to acquire highly sugary and potentially important nutrients while the aphids gain defenses against both predators and parasitoids. Fischer (2005) examined ant and aphid relationships to determine what the most attracts ants to aphids. Honeydew contains several different sugars including tri-, di-, and monosaccharides. The most common sugar found honeydew from *A. fabae* turned was the trisaccharide melezitose, which was the most influential component for aphid husbandry by ants.

In contrast, the relationship between non-ant Hymenoptera and honeydew has been poorly reported. The Mutillidae are a sexually dimorphic, painfully stinging wasp species that prey on other insects. Male mutillids have been reported feeding on honeydew originating from scale insects (Brothers, 1972, 1989; Clouse, et al., 1997). Recently, mutillid males and females were noted to feed on extra-floral nectaries (Luz, et al., 2016). In Greece, honey bees have been occasionally recorded to produce honey from scale honeydew (Santas, 1983). Clouse et al. (1997) recorded diverse hymenopteran families feeding on sand pine aphid, *Pinus clausa*, honeydew. Recorded families were Chrysididae, Colletidae, Eumenidae, Formicidae, Ichneumonidae, Mutillidae, Pompilidae, Rhopalosomatidae, Sphecidae (Crabroninae, Larrinae, Nyssoninae, Sphecinae), Tiphidae, and Vespidae.

Agroecosystems Affecting Hymenoptera

Unlike natural environments, most agricultural systems contain low plant species richness and low genetic diversity in the crop itself. The drastic change of an environment moving from a highly diverse plant community to a singular genetically identical plant species puts pressures on native Hymenoptera as both pollinators and parasitoids require specific ecosystem characteristics. Natural environments with high levels of diversity often support the

Diversity-Stability hypothesis. This hypothesis states that organismal diversity directly confers higher levels of ecosystem stability (Andow, 1991). Expanding on this hypothesis, Andow (1991) correlated predator abundance and Pak et al. (2015) parasitoid abundance with higher levels of polycultures because of the variety of resources available in these systems. Populations of natural enemies had 52.7% more diversity in polycultures over monocultures. Similarly, Risch (1979) found dicultures displayed 75% more species, and 100% more parasitoid diversity than monocultures. Other research has reported the opposite where there was either no difference in diversity or in some cases, a higher diversity in monocultures (Andow & Risch, 1987; Broatch et al., 2010; Vollhardt et al., 2008). Edge effects exhibited by most agricultural systems can remain an oasis for natural predators. Continued research into the diversity dynamics of agroecosystems and wild ecosystems could help to promote native organisms and introduced pollinators.

Macfadyen and Muller (2013) noted that natural enemy abundance was higher along the edges of crop systems and lower further from the edges. Arthropod diversity within agricultural settings also corresponds with diversity in the systems. This can potentially be quantified by surveying fields for parasitoid diversity (Anderson et al., 2011) because parasitoid diversity should correspond with greater diversity of hosts.

Enhancing agricultural settings to attract pollinators benefits both the natural and agricultural environments. Pollinators contribute approximately \$3 billion annually to agriculture through increased yield (Smith et al., 2008) and crop quality (er al., 2012). Cover crops enable beneficial pollinators to use the resources within fields and promote their immigration. This then results in better yield and healthier plants. For example, bumble bees (*Bombus* spp.) have been shown to increase in abundance when mass flowering cover crops are planted (Westphal et al., 2003).

Planting cover crops does more than enhance diversity of beneficial insects (Altieri & Schmidt, 1986; Lavandero et al., 2005), as it also contributes to abiotic effects as well. Wratten et al. (2012) demonstrated that improving ecosystems for pollinators mitigates erosion, improves natural enemy diversity and abundance, improves water quality, and promotes favorable nutrient cycling patterns. Supporting these observations, Barel et al. (2018) showed that winter cover crops improve nitrogen levels within the soil. Fertilizers have been shown to affect pest and predator populations mostly due to the efficiency the pests can gain from the increased level of micronutrients. Fertilizer has been shown to promote the reproduction rate of pest species like aphids, which then promotes the abundance of their biological controls (Ali, et al., 2013).

Sampling Hymenoptera

The order Hymenoptera is among the most diverse groups of insects. These insects exhibit a multitude of life histories and behaviors, serving as predators, parasitoids, herbivores, and pollinators (LaSalle et al., 1993). Numerous methods exist for sampling insects, including active methods such as aerial nets and passive methods such as pitfall traps. Pan traps are easily deployable traps that are normally colored blue, yellow, or white and are attractive to insects that visit flowers. The traps, which is normally a bowl or cup, hold soapy water and are placed on the ground. Attracted insects land and then sink. Aerial net collecting is usually used for large species that may be predators or pollinators that can escape pan traps (Roulston et al., 2007). These larger species may also not be attracted to pan traps and thus, elude capture (Roulston et al., 2007). Window traps are similar to pan traps, but use clear panels to intercept flying insects. When the flying insect hits the panel it falls into soapy water and drowns. These traps are most commonly used in forest settings being suspended from or fixed to tree trunks. Normally they are used to collect beetles living in dead wood, however, reports of diverse insect fauna being

collected from them pushed researchers to explore this phenomena (Rubene et al., 2015). Among common trapping methods, yellow sticky card traps are used to collect smaller insects including parasitoids (Dowell et al., 1983). These traps are normally placed at plant height, attract insects because of their color, and capture them with sticky adhesive (Dowell et al., 1983). Like pan traps, larger species are often strong enough to escape from the adhesive.

Pan traps have been noted to attract the widest range of species, especially rare species that may not be collected from net or sticky card traps (Roulston, Smith, & Brewster, 2007; Shrestha et al., 2019; Tuell, Ascher, & Isaacs, 2009). When pan traps and window traps were compared, no difference was found in species diversity; however, window traps were good at collecting wood-nesting bees while pan traps were superior for collecting pollinators (Rubene, et al., 2015). Intensive net sampling has been shown to be more efficient in collecting larger species such as bumble bees, which are not highly attracted to pan traps (Roulston et al., 2007). Dowell and Cherry (1981) researched parasitoid survey methods using different colored sticky cards and determined yellow sticky cards were the most effective.

Thesis Objectives

- 1) Investigate sorghum lines for resistance to SCA using screen trials conducted on sorghum seedlings in a greenhouse.
- 2) Characterize Hymenoptera diversity and abundance associated with SCA infestations in sorghum and Johnson grass using pan traps and sticky traps.

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CHAPTER II

IDENTIFICATION OF INDUSTRY SORGHUM GERMPLASM RESISTANCE TO SUGARCANE APHID, *MELANAPHIS SACCHARI*

Abstract

Since 2013, the sugarcane aphid, *Melanaphis sacchari*, has been a perennial pest of grain sorghum throughout the central United States. Sorghum germplasm have been developed that are resistant to sugarcane aphid, but additional tests may reveal better germplasm or allow production of hybrids with other desirable traits. Grain sorghum seedlings were screened for resistance to sugarcane aphid using germplasm obtained from three companies. After exposure to sugarcane aphid, germplasm were rated for damage, chlorophyll content, plant height, and number of leaves and were compared to known resistant and susceptible lines. Fifteen of the tested germplasm exhibited resistance based on damage rating and chlorophyll content; however, many had reductions in height or leaf number. This study furthers knowledge of sorghum germplasms that are resistant to sugarcane aphid feeding.

Key Words: Plant resistance, grain sorghum, piercing-sucking damage, tolerance

Introduction

Sorghum, *Sorghum bicolor* [L.] Moench, is the world's fifth most-important grain in terms of production and acreage. Sorghum grows in hot, arid agro-ecosystems that are frequently drought prone and characterized by generally poor soil quality where most other food grains do not survive. In the semi-arid tropics, sorghum is a vital source of food for millions of people (House, 1985). It also provides an important source of raw material for many uses including forage for animals, sugar for drinks and biofuels, and for fiber.

Sorghum has more than 130 documented pests, but pest severity differs depending on the crop locality (Reddy & Davies, 1979). North America, where sorghum was introduced, had relatively few serious pests of grain sorghum until 2013 when the sugarcane aphid (SCA) (*Melanaphis sacchari* Zehntner) was discovered colonizing it (Sharma, 1993). Until then, SCA had only been a pest in the continental United States on sugarcane in Florida and Louisiana (Mead, 1978). This host plant change and subsequent widespread damage surprised growers and scientists, resulting in genetic studies focused on evaluating SCA from across the world. Nibouche et al. (2014, 2015, 2018) analyzed the genetic makeup of SCA from different populations, but only six different superclone lineages were identified. The aphids found on sorghum did not belong to any previous superclone lineage, which meant SCA on sorghum represent a different biotype than was previously known.

The SCA has now become one of the most destructive sorghum pests in the United States. The aphid was originally reported on sorghum in South Texas in 2013, and later would be discovered in three other states as well as northern Tamaulipas, Mexico (Rodríguez-del-Bosque & Terán, 2015). Only two years later SCA was found in 17 states and 400 counties (Bowling et al., 2016). Within Texas alone, SCA caused \$276.17 million (U.S. dollars) of losses in sorghum during the 2014-2016 growing seasons (Zapata et al., 2018). Yield loss percentages caused by

SCA in Mexico ranged from 30-100% (Rodríguez-del-Bosque & Terán, 2015). These losses are from direct feeding and are compounded when SCA transmits diseases including sugarcane yellow virus, millet red leaf virus, and mosaic potyvirus leaf (Akbar et al., 2010).

Many different control strategies have been used to combat SCA in sorghum, but one of the most desirable methods is identifying host plant resistance (Painter 1958) This method has been used around the world, and regularly used in order to combat pest problems in agriculture. Resistant sorghum has been developed in Japan and India against SCA as they continue to experience trouble with this pest (Hagio, 1992; Sharma et al., 2013, 2014). Similarly, the USDA has developed and tested known resistant cultivars (Armstrong et al. 2015, 2017, 2018). Some of the cultivars were further examined to determine which type(s) of resistance they might express against SCA. Limaje et al. (2018) documented both antibiosis and tolerance qualities, outlining proper procedures to determine both types of host plant resistance.

The continued development of resistant cultivars is essential for the success of the sorghum industry. Over time, pests may overcome resistance traits and new cultivars must be developed to match growing conditions and to improve yield. We tested industry-developed grain sorghum germplasm and compared them to known resistant and susceptible cultivars.

Methods

Resistance levels of sorghum germplasm from three private companies were determined using a singular sugarcane aphid genetic line. The use of an isolated genetic line allows for a consistent damage rating when comparing plants with resistance and susceptible traits. The SCA originated from a single gravid female collected from Matagorda County, TX, in August 2013. This female was collected from a field of grain sorghum, which had been colonized by SCA. Since then, a colony has been cultured and maintained at the USDA-ARS station in Stillwater,

Oklahoma. This SCA colony is maintained on susceptible ‘RTx7000’ sorghum in 4.4-L pots fitted with cages consisting of 45-cm tall × 16-cm diameter cylinders of Lexan™ (SABIC Polymershapes, Tulsa, OK) topped with organdy cloth that allows ventilation while preventing aphids from escaping. Every two weeks the aphids are transferred onto new plants that are maintained in a greenhouse at 21°C to 31°C and with two T-8 fluorescent lights to ensure a minimum of 16 h of light.

Nineteen germplasm lines were compared with four germplasm checks. The check germplasms used were the known resistant ‘TX2783’, and three known susceptible lines: ‘TX7000’, ‘KS585’, and ‘WSH117’. A free-choice flat screen trial was used to examine aphid damage on sorghum seedlings. Plant response to SCA feeding was measured as plant height, chlorophyll content, and leaf number. For experimental trials (N = 3), we also planted control flats (N = 2) with the same germplasm. When constructing flats, 15 replicates of each cultivar were randomized using Research Randomizer (2016).

For experimental flats, aphids were introduced to the flats by laying heavily infested sorghum (‘TX7000’) leaves down each column and across each row (Starks and Burton 1977). Evaluation of the flats occurred when the known susceptible germplasms (‘TX7000’, ‘WSH117’, ‘KS585’) were approximately 90-100% dead. The plants in both the control and experimental flats were evaluated for damage on a 1-9 scale where a 1 denotes a plant that is visually undamaged, a 2 represents a plant with 1-5% chlorotic tissue, a 3 equals 5-20% and 4 21-35%; 5, is 36-50%; 6 is 51-65%; 7 is 66-80% is 8, 81-95% and 9 equates to 96-100% chlorosis or a dead plant (Webster et al. 1991, Burd et al. 2006).

After assigning damage ratings, the plants were measured for height by cutting the plants at the soil level, laying the plant on a ruler, extending its leaves out, and recording the entire length (cm). The difference in plant height was calculated by subtracting the length of damaged

plants from the length of checks. The number of leaves were counted and a SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ), which sums both a and b chlorophyll types (Markwell et al., 1995), was used to estimate chlorophyll content. Chlorophyll data were collected by taking one reading at three different spots on different leaves when possible. Values were then averaged to represent mean chlorophyll content. The chlorophyll loss index was calculated by comparing control and experimental plants of the same germplasm $(C-T)/C*100$ where C is the mean chlorophyll reading from SPAD in the control flat, and T is the mean chlorophyll from the plants exposed to aphids.

Response variables (plant height and chlorophyll content) for both the control and experimental flats were compared using a general linear model one-way analysis of variance. Significance was determined using the least-squares means pairwise comparisons procedure. Leaf number was compared using Dunn's multiple comparison test in order to determine the germplasm relatedness when compared to the known susceptible and resistant varieties. Plant damage was analyzed using a non parametric pairwise analysis (Dunn's test) to compare the entry mea with the same alpha value and incorporating the least-squared pairwise comparisons (SAS users guide, version 9.3, 2010).

Results

A number of the tested varieties compared favorably to known resistant cultivars when exposed to feeding by sugarcane aphids. Damage ratings were similar among tested cultivars and significantly lower than for known susceptible varieties (Table 1). Chlorophyll content after aphid feeding was highest in SPSA203 and SPSA302 while plant height was most similar between damaged and check plants for these same varieties (Tables 1, 2). The remaining cultivars all performed as well as the known resistant cultivar 'TX2783' based on damage ratings when

exposed to aphid feeding (Tables 1, 2). ‘Bayer Check 2’ and ‘Channel 5R45’ had the highest chlorophyll content of the tested varieties. The latter variety also had the least difference in height and the most leaves compared to other tested varieties (Table 2). Among the tested germplasms, two varieties, ‘W-625Y’ and ‘W-902W’ were found to be highly susceptible to sugarcane aphid feeding damage, while ‘W-7721’ was found to exhibit similar resistance as the known resistant variety ‘TX2783’ (Tables 1, 2).

Discussion

The tested germplasm from all three companies displayed a range of resistance to feeding by sugarcane aphids. Varieties that display <3 damage rating exhibit high levels of resistance, and ratings of 3-4 indicate resistance (Armstrong et al. 2015). Susceptible cultivars have damage ratings of 6-7 or higher. From the tested varieties, ‘SPSA203’, ‘SPSA204’, ‘SPSA301’, ‘SPSA302’, ‘SPSA306’, ‘SPX17617’, ‘Bayer Check 2’, ‘Channel 5R45’, ‘Channel 7B20’, ‘Dekalb DKS54-07’, ‘Fontanelle G6008’, ‘Fontanelle G3802’, ‘W-5911’, ‘W-7706W’, and ‘W-7721’ exhibited similar resistance to the known resistant variety ‘TX2783’ (Tables 1, 2).

Plant resistance to aphid feeding can be in the form of tolerance to damage, antibiosis, or antixenosis (Painter 1958). The flat screen trials conducted in this study do not allow determination of the type of resistance because aphids could choose to colonize specific cultivars during infestation and because aphid reproduction was not monitored or quantified. However, measures of chlorophyll content, leaf number, and plant height relative to uninfested controls suggest plant tolerance for some of the tested cultivars. The varieties that maintained the highest chlorophyll content were ‘W-7721’, ‘Channel 5R45’, and the five SPSA varieties may suggest plant compensation to feeding. Higher chlorophyll content also correlated with maintenance of plant growth, suggesting that aphid feeding did not disrupt assimilation of photosynthate.

In the current study of grain sorghum seedlings, ‘SPSA203’, ‘SPSA204’, ‘SPSA301’, ‘SPSA302’, ‘SPSA306’, ‘SPX17617’, ‘Bayer Check 2’, ‘Channel 5R45’, ‘Channel 7B20’, ‘Dekalb DKS54-07’, ‘Fontanelle G6008’, ‘Fontanelle G3802’, ‘W-5911’, ‘W-7706W’, and ‘W-7721’ were resistant to feeding by SCA. Measures of chlorophyll content support the conclusion of resistance because the two values (damage rating and chlorophyll content) were closely linked as documented from Girma et al. (1998). Plant height and leaf number resulted in more ambiguous results as some cultivars expressed susceptible values when compared to checks even though damage rating and chlorophyll content designated them as resistant.

Future work should focus on both evaluating the mode of plant resistance and field tests to determine yield of the varieties. Additionally, plant response to different growing conditions should be evaluated.

Table 1: The Median and Mean (\pm S.E.) for damage rating and chlorophyll content, respectively, for the germplasm exposed to sugarcane aphid compared to checks and known resistant and susceptible varieties.

Germplasm	Damage Rating	Chlorophyll Content
Bayer Check 1	9 a	4.6 \pm 2.7 d
Bayer Check 2	5 bc	29.1 \pm 2.5 a
Channel 5R45	4 bc	28.8 \pm 3.1 a
Channel 7B20	7 ab	18.0 \pm 4.5 bc
Dekalb DKS54-07	6 b	17.1 \pm 3.8 bc
Fontanelle G3802	5 bc	22.7 \pm 3.9 ab
Fontanelle G6008	6 ab	22.1 \pm 4.0 ab
Gro-N-Graze Defender	8 a	4.5 \pm 3.1 d
KS585**	9 a	8.8 \pm 3.9 cd
SPSA203	3 c	28.8 \pm 3.1 a
SPSA204	5 bc	25.5 \pm 3.6 ab
SPSA301	5 bc	27.4 \pm 3.0 a
SPSA302	5 bc	26.4 \pm 3.7 a
SPSA306	7 ab	24.5 \pm 3.6 ab
SPX17617	7 ab	15.7 \pm 4.0 bc
TX2783*	7 ab	22.5 \pm 3.9 ab
TX7000**	9 a	2.1 \pm 2.1 d
W-5911	6 b	19.2 \pm 4.3 abc
W-625Y	9 a	5.8 \pm 3.1 d
W-7706W	4 bc	21.0 \pm 4.1 ab
W-7721	4 bc	24.9 \pm 4.1 a
W-902W	9 a	9.4 \pm 3.6 cd

WSH117**

7 ab

11.2 ± 3.8 cd

Damage Rating: df = 9, 134, F = 13.08 P > F = 0.0001

Chlorophyll Content: df = 9, 140, F = 6.93 P > F = 0.0001

* Known resistant check

** Known susceptible check

Table 2: Mean (\pm S.E.) for plant height and leaf number for germplasm exposed to sugarcane aphid compared to checks and known resistant and susceptible varieties.

Germplasm	Difference in Plant Height	Leaf Number
Bayer Check 1	19.6 \pm 1.6 ab	1.8 \pm 0.4 b
Bayer Check 2	12.3 \pm 1.9 cd	2.7 \pm 0.6 ab
Channel 5R45	10.7 \pm 1.9 d	3.0 \pm 0.7 a
Channel 7B20	18.1 \pm 1.8 b	2.5 \pm 0.6 a
Dekalb DKS54-07	16.7 \pm 1.9 bc	2.8 \pm 0.6 a
Fontanelle G3802	15.5 \pm 2.2 bcd	2.6 \pm 0.7 a
Fontanelle G6008	18.6 \pm 1.6 ab	2.1 \pm 0.8 ab
Gro-N-Graze Defender	12.5 \pm 1.6 c	2.3 \pm 0.5 a
KS585	16.0 \pm 1.8 bcd	2.4 \pm 0.7 a
SPSA203	11.0 \pm 1.8 d	2.9 \pm 0.7 a
SPSA204	11.9 \pm 2.3 d	2.9 \pm 0.7 a
SPSA301	15.9 \pm 1.6 bcd	2.7 \pm 0.6 a
SPSA302	11.4 \pm 2.4 d	2.4 \pm 0.7 a
SPSA306	11.90 \pm 1.8 d	2.4 \pm 0.7 a
SPX17617	17.2 \pm 1.6 bc	2.3 \pm 0.6 a
TX2783*	12.4 \pm 1.8 cd	2.6 \pm 0.8 a
TX7000**	23.2 \pm 1.1 a	2.2 \pm 0.4 a
W-5911	14.4 \pm 2.2 bc	2.5 \pm 0.8 a
W-625Y	18.9 \pm 1.5 ab	2.1 \pm 0.4 ab
W-7706W	12.3 \pm 2.1 c	2.7 \pm 0.7 ab

W-7721	14.5 ± 2.0 bc	2.9 ± 0.8 a
W-902W	14.6 ± 1.4 bc	2.1 ± 0.7 ab
WSH117**	20.2 ± 1.0 ab	2.1 ± 0.6 ab

Difference in Plant Height: df = 9, 134, F = 5.14 P > F = 0.0001

Leaf Number: df = 9, 134, F = 2.23 P > F = 0.0240

* Known resistant check

** Known susceptible check

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CHAPTER III

HYMENOPTERA FEEDERS: SUGARCANE APHID (*MELANAPHIS SACCHARI*) HONEYDEW AS A RESOURCE IN SORGHUM (*SORGHUM BICOLOR*) AND JOHNSONGRASS (*SORGHUM HAPLENESE*)

Abstract

The sugarcane aphid (SCA), *Melanaphis sacchari*, a recent perennial pest of sorghum, quickly forms dense colonies and produces large amounts of waste material commonly called honeydew. The aphids normally feed on the underside of leaves as well as the stalk, resulting in honeydew accumulation on the leaf directly below them and allowing secondary growth of sooty mold fungi. While aphid colonization and sooty mold growth can cause economic loss, honeydew may create a resource that other insects could use, especially in areas that have few plants in the flowering stage. A pan trap and yellow sticky card survey was conducted in sorghum and Johnsongrass sites where SCA was found. In these surveys, blue, yellow, and white pan traps were used along with yellow sticky traps placed on a garden stake at plant height. Sixteen sorghum sites with aphids and four control sites were surveyed along with 12 sites with Johnsongrass. Nearly 4,000 Hymenoptera belonging to 31 families and 121 morphospecies were collected. Of these, 84% were collected from fields with sugarcane aphid infestations. Future research should examine whether strips with susceptible sorghum at field edges may

benefit Hymenoptera that are predators, parasitoid, and pollinators by encouraging sugarcane aphids that provide honeydew as a sugar source.

Introduction

Honeydew, the waste excretia of hemipteran plant feeders, often accumulates on host plant leaves as the population grows. This substance seemingly would be void of nutrients due to its origins; however, molecular analysis shows how honeydew is actually a diverse concoction of many different proteins, sugars, and free amino acids (Fischer, 2005; Sabri et al., 2013). Honeydew creates a beneficial medium for sooty mold to grow, further hindering the plant's photosynthetic potential (Bowling et al., 2016), especially when fluid-feeding insects reach high densities.

The sugarcane aphid (SCA) (*Melanaphis sacchari*) is a prolific plant feeder that multiplies exponentially and produces abundant honeydew. This pest is thought to have originated from Asia; however, its current range encompasses most continents (Singh, et al., 2004). Genetic analysis has identified distinct biotypes (Nibouche et al., 2018; Nibouche et al., 2014), which can be correlated to host plant preferences. In Texas in 2013, SCA was found feeding on sorghum as opposed to its documented host in North American, sugarcane (Mead, 1978; Bowling et al., 2016). On sorghum, the SCA biotype displays high feeding activity and reproduction and under favorable conditions, high infestations consisting of tens of thousands of aphids per leaf may occur. The feeding also results in honeydew accumulation, causing a glossy appearance on leaves.

With a conservative estimate of one million species, hymenopteran species occupy most niches (Ulrich, 1999). Hymenoptera can most often be categorized as pollinators, parasitoids, or predators, although some groups including ants can be omnivores/ detritivores. Adults require sugars as nutrients and many species will seek nectar. Some ants (Formicidae) are known to tend

aphid or scale colonies, creating a mutualistic relationship where the ants protect the aphids and scales from predators and parasitoids and, in turn, receive sugars from honeydew (Nixon, 1951).

Fischer (2005) studied ant husbandry in a mutualistic relationship with *Aphis fabae* and identified the trisaccharide, melezitose. As the concentration of melezitose in honeydew increased it correlated with greater instances of husbandry. In addition to ants, some parasitoid species in agricultural systems are known to take advantage of honeydew resources (Wäckers et al., 2008). Not only can the honeydew excreted by a host species supplement the parasitoid diet, but it can act as a kairomone, allowing for the parasitoid to locate its host (Wäckers et al., 2008). Other Hymenoptera may also form relationships with aphids and honeydew. The Apoidea pollinate plants as they visit flowers in search for pollen and nectar resources increasing plant production (Brittain, Williams, Kremen, & Klein, 2013; Calderone, 2012). Surprisingly, little documentation of native bees utilizing alternatives to flower nectar exists.

In most large-scale agricultural systems, crops are planted as monocultures with plants that are similar genetically and have nearly simultaneous development. Unfortunately, native and introduced pollinators often suffer from a lack of floral sources throughout the year. Extensive research has documented population reductions on native fauna when floral sources are scarce. The majority of findings show that a diversified plant community supports far more natural enemies and pollinators (Altieri & Schnidt, 1986; Andow, 1991; Wretten et al., 2012)); however, for specific plant pests and their respective natural enemies the relationship can vary (Volhardt et al., 2008).

In 2018, I observed diverse Hymenoptera in sorghum fields infested by SCA with many species visiting the aphids and the area of accumulated honeydew. I hypothesized that SCA and its honeydew serve as a resource for Hymenoptera and that Hymenoptera numbers and diversity would increase in infested fields.

Methods

Sorghum:

Initial observations in sorghum fields of Hymenoptera feeding on honeydew excreted from the SCA occurred in 2018. To experimentally test the effect of honeydew on Hymenoptera occurrence, plots were established at the Cimarron Valley Research Station located near Perkins, OK and in Stillwater, OK. One plot was established as an experimental plot with SCA while the other served as a control. The fields were approximately 0.48 km apart.

In the experimental field four rows of susceptible cultivars were selected and each row was flanked by a resistant cultivar allowing for 3.05 meters of space between each experimental row. Four locations per row were selected which were 10 meters apart. Three different colors of pan traps (blue, yellow, and white) consisting of 350-ml (12 oz) plastic bowls were used to sample Hymenoptera. The traps were half filled with soapy water (Dawn dish soap and water) and placed in a triangle. Along with pan traps, yellow sticky card traps (Alpha Scents, Inc.: yellow card-double sided, 8 x 5.5in) were placed at every location. These traps were attached to garden stakes with clothes pins set approximately at plant height.

Sampling occurred between 800 hours and 1930 hours when both bowls and sticky cards were placed. When traps were checked, the contents of each bowl were poured through a strainer and collected insects were placed in a labeled 50-ml plastic vial with 70% ethyl alcohol. Sticky card traps were placed in labeled one-gallon plastic bags. For controls, four sampling locations were randomly chosen in the field that had only the resistant cultivar and sampling protocols were the same as above.

After collection, the pan trap contents were stored in vials. The Hymenoptera from pan traps were sorted, washed, voucher specimens were pinned, labeled, and classified to morphospecies.

Johnsongrass:

The effects of SCA presence on Hymenoptera was also tested in Johnsongrass. Plots were chosen at Cimarron Valley Research Station, Efaw, a small farm owned by Oklahoma State in Stillwater, OK and at the Insect Adventure also in Stillwater. These sites were selected because SCA colonies were found on the Johnsongrass. In total there were seven trap locations at the Cimarron Valley Research Station, four at Efaw, and one at the Insect Adventure. One site at the Cimarron Valley Research Station acted as a control, and the rest were experimental sites.

Analysis

To examine the effects of aphids on Hymenoptera families, I used chi-squared goodness of fit tests with significance being judged as $P < 0.01$. This analysis compares the number of individuals collected from the experimental and control sites highlighting a significant difference from the resulting value. To examine the effects of aphids on overall diversity, results from the plants with SCA were compared to controls using the Shannon-Wiener Diversity and Simpson's Diversity indices. Shannon-Weiner Diversity measures overall biological diversity of samples while the Simpson's Index measures evenness (Yeom & Kim 2011).

Results:

A total of 3,950 Hymenoptera were collected during this study, 2,384 individuals from sorghum, and 1,566 from Johnsongrass. There were 30 families and 124 morphospecies collected from sorghum and 29 families and 121 morphospecies from Johnsongrass.

The presence of SCA strongly influenced the number of Hymenoptera collected in both sorghum and in Johnsongrass. In sorghum, 1,892 Hymenoptera were collected from fields with SCA compared to 484 from check plots. The presence of aphids significantly increased the number of ants, halictid bees, and scelionid wasps. Aphids also significantly increased the presence of sphecid and braconid wasps. Aphids did not influence the presence of pompilids or mutillids (Table 1). In Johnsongrass, 1,490 Hymenoptera were collected from plots with SCA and only 74 from check plots. In Johnsongrass, aphids increased the numbers of more groups of Hymenoptera, including braconid wasps and pompilids (Table 2).

Pan traps collected more individual Hymenoptera than sticky traps (2,588 compared to 1,363 respectively) with about 1. times more individuals in pan traps placed in sorghum and about twice as many for pan traps placed in Johnsongrass (Table 3). The highest number of morphospecies (100) were collected in pan traps in sorghum and the lowest number were collected from sticky traps placed in Johnsongrass (65). Both crop type and trap type influenced captures of Hymenoptera. Families that were most abundant in sorghum were Halictidae (N = 638), Scelionidae (N = 466) and Formicidae (N = 613). In Johnsongrass, Formicidae (N = 801) and Scelionidae (N = 312) were most commonly collected. Pan traps collected more specimens of larger species (Apoidea, Pompiloidea, and Formicoidea) compared to sticky traps that collected smaller parasitic species (Scelionidae, Platygasteridae, and Mymaridae) (Table 3).

Diversity indices were higher for Hymenoptera collected in sorghum with SCA compared to sorghum alone for the majority of samples and the diversity increases through the duration of the experiment (Table 4). Simpson index values that are close to 1.0 represent dominance by one or a few species with the majority of the remaining organisms being represented by one or few individuals. Thus, Shannon-Wiener diversity for sorghum was moderate but overall evenness of diversity (Simpson diversity index) was poor (Table 4). In contrast to the results obtained from sorghum, the Shannon-Diversity indices of Johnsongrass were always greater for plots with SCA.

The Simpson index values indicate that the evenness of morphospecies was low and overall; however, the loss of control plots limited the ability to interpret diversity (Table 4).

Discussion

Through this study, I examined whether SCA feeding and resulting honeydew attract Hymenoptera that may use the SCA waste products as a resource. I found that traps placed in fields of sorghum and Johnsongrass with SCA collected substantially more Hymenoptera (Tables 1-3) than trap sin fields without SCA. Caution in interpreting the results is required because there were more samples taken from fields with aphids than from control sites, especially with Johnsongrass where a number of sites were lost during the study. The sites were lost due to mowing, herbicide spraying, or flooding. In addition, results from using morphospecies instead of species-level identification should be cautiously interpreted because sex differences could lead to over-representation of apparent species while morphologically similar specimens could represent cryptic species. Future studies should take place in Johnsongrass monocultures and potentially use control methods to prevent the spread of SCA into control plots. Despite the limitations of the current data, documentation of substantial increases in diverse Hymenoptera strongly support that additional research is warranted.

Previous research has documented ant use of hemipteran waste products and in this study three morphospecies of ants were collected from sorghum and 11 morphospecies from Johnsongrass. Almost all ants ($N = 1,404$) were collected from pan traps compared to sticky traps ($N = 16$). Because ants are all eusocial insects that forage collectively, their influence on diversity and total number of Hymenoptera collected should also be viewed with caution. From my study, ant diversity in sorghum appears limited compared with the Johnsongrass sites, which were placed on the field edges away from the managed agroecosystems. The difference in the number of morphospecies collected support this observation ($N=3$ in sorghum, $N=11$ in Johnsongrass).

In contrast with ants, flying solitary Hymenoptera provide a stronger indication of the influence of SCA on diversity. These Hymenoptera can be attracted to the sugar resource, to prey, or to hosts. It is difficult to delineate the exact reasons for the attraction of groups such as Sphecidae that are predators or Scelionidae that are parasitoids. Because all flying Hymenoptera require sugars to fuel flight, it could be assumed that they are taking advantage of the resource; however, it is unclear whether they favor or even benefit from consuming honeydew. Parasitoids have been known to increase in diversity and abundance as prey organisms increase in prevalence (Anderson et al., 2011). These species may respond to SCA for both nutrients or breeding resources. The Apoidea, which are nectar and pollen feeders, provide the clearest example of a positive influence of SCA honeydew on Hymenoptera. The collection of 12 times more Halictidae in plots with SCA in sorghum and 26 times more Halictidae in Johnsongrass with SCA supports the conclusion that the honeydew produced by SCA is being used by Halictidae.

The trapping methods used in this study did not capture all of the observed Hymenoptera diversity, especially for larger species. During the initial observations which spawned this research, much larger vespoids, sphecids, and more diverse mutillids were observed feeding on the honeydew. Future research should focus on creating transects within sorghum and Johnsongrass plots in order to net larger Hymenoptera species. Active netting of individuals would ensure that the larger Hymenoptera not normally captured with pan traps could be evaluated (Roulston, Smith, & Brewster, 2007). In this study, I captured relatively few European honey bees (*Apis mellifera*) despite regularly observing good numbers visiting aphids. Large strong species including *Pepsis* spp., tarantula hawk wasp, or the cicada killer, *Sphecius speciosus*, were observed in the fields but never captured in pan traps or on sticky traps. In addition, yellow sticky traps were chosen over a different color because they have been found to

be the most effective when sampling parasitoids (Dowell & Cherry, 1981). However, some species are attracted to blue cards.

Sorghum, like most grasses does not produce nectar, and thus, commercial sorghum fields provide few apparent resources for Hymenoptera. Areas with Johnsongrass, a weedy invasive species likewise provide few apparent resources as it forms dense monocultures. In contrast to expectations, Hymenoptera diversity in areas with these grasses was moderately high, though strongly influenced by a few common species (Table 4). When SCA was present, the number of individual Hymenoptera increased and there was a trend of increasing diversity for the duration of this 6-week study. Between late July and September, floral resources may become more scarce with high temperatures and lack of rainfall. The SCA honeydew could play a part in maintaining some Hymenoptera species.

Overall Conclusions

The majority of contemporary research supports the hypothesis that as plant communities diversify, the fauna also diversifies (Andow 1991, Pak et al. 2015). While some research shows little or the opposite affect (Vollhardt 2008). Not only does diversity improve with a diverse plant community, but crop health and yield also benefits from plant diversity (Smith, Gross, & Robertson, 2008). Crop diversity benefits natural enemies that may provide pest control in diverse agroecosystems (Andow 1991) . In addition, diverse cover crops improve plant biomass and nutrient availability within the soil even after the cover crops are gone from the system (Barel et al., 2018). Despite these documented benefits, large-scale monocultures are widely planted because of efficiencies gained in cultivation and harvest. Finding novel ways to provide ecosystem benefits promoting native organism populations are critical to sustainable production.

Calderone (2012) estimated the economic value of pollinators for U.S. agricultural systems to be \$15.12 billion (2009) and \$12 billion (2004) for direct and indirectly pollinated crops respectively. Much of the value of pollination is attributed to the management of the European honey bee. Honey bees are documented to collect pollen from sorghum, but this is the first experimental evidence of honey bees also collecting honeydew from an invasive pest that forms large colonies and produces large quantities of waste. Future research should examine if SCA could be managed to provide resources to benefit both managed bee hives and native biodiversity by planting a strip of susceptible sorghum at the field edge, and planting the rest of the field with a resistant cultivar.

Table 1: Comparison of Hymenoptera families and morphospecies collected in Sorghum infested with sugarcane aphids and un-infested checks using pan traps and sticky cards.

Superfamily	Family	Sorghum			
		With Aphids		Without Aphids	
		# of Morphospecies	# of Individuals	# of Morphospecies	# of Individuals
Apoidea	**Halictidae	12	618	6	48
	Apidae	9	10	1	1
	Andrenidae	1	5	1	1
	*Sphecidae	19	41	4	6
	Megachilidae	0	0	0	0
Pompiloidea	Pompilidae	8	34	5	37
	Mutillidae	1	4	0	0
Chrysoidea	Chrysididae	1	1	0	0
	Bethylidae	1	5	2	8
	Dryinidae	1	1	0	0
Tiphioidea	Tiphiidae	1	7	1	3
	Sierolomorphidae	1	1	0	0
Ichneumonoidea	Ichneumonidae	2	2	1	1
	*Brachonidae	7	17	2	3
Chalcidoidea	Aphelinidae	2	5	1	1
	Chalcidae	3	7	0	0
	**Encyrtidae	6	89	5	26
	Eupelmidae	1	1	1	1
	Eurytomidae	2	3	0	0
	**Mymaridae	4	48	4	9
	Pteromalidae	7	26	1	50
	Torymidae	1	6	1	3
	Eulophidae	2	3	0	0
	Perilampidae	1	1	1	1
Cynipoidea	Figitidae	4	17	1	5
Diaprioidea	*Diapriidae	2	15	1	1
Ceraphronoidea	Ceraphronidae	1	2	1	1
Platygastroidea	Platygastridae	3	13	2	11
	**Scelionidae	12	376	8	179
Formicoidea	**Formicidae	3	528	3	85
Evanoidea	Evaniidae	0	0	0	0
Unknown		3	6	3	3
TOTALS		121	1,892	56	484

* Chi-squared goodness of fit $P < 0.01$

**Chi-squared goodness of fit $P < 0.001$

Table 2. Comparison of Hymenoptera families and morphospecies collected in Johnsongrass infested with sugarcane aphids and un-infested checks using pan and sticky traps.

Superfamily	Family	Johnsongrass			
		With Aphids		Without Aphids	
		# of Morphospecies	# of Individuals	# of Morphospecies	# of Individuals
Apoidea					
	**Halictidae	13	78	3	3
	Apidae	2	2	0	0
	Andrenidae	3	3	0	0
	*Sphecidae	6	10	0	0
	Megachilidae	1	1	0	0
Pompiloidea					
	*Pompilidae	9	16	2	1
	Mutillidae	2	16	0	0
Chrysoidea					
	Chrysididae	1	1	0	0
	Bethylidae	4	9	1	7
	Dryinidae	0	0	0	0
Tiphioidea					
	Tiphiidae	1	4	0	0
	Sierolomorphidae	0	0	1	1
Ichneumonoidea					
	Ichneumonidae	6	9	0	0
	*Brachonidae	6	12	2	2
Chalcidoidea					
	Aphelinidae	1	1	0	0
	Chalcidae	3	4	0	0
	**Encyrtidae	6	31	0	0
	Eupelmidae	2	2	0	0
	Eurytomidae	1	3	0	0
	**Mymaridae	6	36	3	4
	**Pteromalidae	3	7	2	2
	Torymidae	1	2	0	0
	Eulophidae	0	0	0	0
	Perilampidae	0	0	0	0
Cynipoidea					
	**Figitidae	3	22	1	1
Diaprioidea					
	Diapriidae	3	6	1	1
Ceraphronoidea					
	Ceraphronidae	2	33	0	0
Platygastridae					
	Platygastridae	2	19	0	0
	**Scelionidae	13	365	3	23
Formicoidea					
	**Formicidae	11	789	5	28
Evanoidea					
	Evaniidae	1	1	0	0
Unknown					
		4	8	1	1
TOTALS		116	1,490	25	74

* Chi-squared goodness of fit $P < 0.01$

**Chi-squared goodness of fit $P < 0.001$

Table 3. Comparison of trapping method in sorghum and Johnsongrass.

Superfamily	Family	Sorghum				Johnsongrass			
		Pan		Sticky		Pan		Sticky	
		Types	#	Types	#	Types	#	Types	#
Apoidea	Halictidae	12	638	5	28	9	68	6	13
	Apidae	9	11	0	0	2	2	0	0
	Andrenidae	1	6	0	0	3	3	0	0
	Sphecidae	19	42	3	5	5	10	0	0
	Megachilidae	0	0	0	0	1	1	0	0
Pompiloidea	Pompilidae	9	64	1	7	12	18	0	0
	Mutillidae	1	2	1	2	4	12	1	4
Chrysoidea	Chrysididae	1	1	0	0	1	1	0	0
	Bethylidae	1	3	2	10	3	5	2	11
	Dryinidae	1	1	0	0	0	0	0	0
Tiphioidea	Tiphiidae	1	7	1	3	1	1	1	3
	Sierolomorphidae	1	1	0	0	0	0	1	1
Ichneumonoidea	Ichneumonidae	1	1	2	2	5	7	2	2
	Brachonidae	4	7	5	19	3	3	3	11
Chalcidoidea	Aphelinidae	1	2	1	4	1	1	0	0
	Chalcidae	2	6	1	1	0	0	3	4
	Encyrtidae	4	9	6	107	1	4	6	28
	Eupelmidae	1	1	1	1	2	3	0	0
	Eurytomidae	1	1	2	2	0	0	1	3
	Mymaridae	1	2	4	55	0	0	6	40
	Pteromalidae	7	8	6	68	2	2	4	7
	Torymidae	0	0	1	10	0	0	1	2
	Eulophidae	2	2	2	1	0	0	0	0
	Perilampidae	0	0	1	2	0	0	0	0
Cynipoidea	Figitidae	3	11	3	11	3	7	2	16
Diaprioidea	Diapriidae	2	12	2	4	3	5	2	2
Ceraphronoidea	Ceraphronidae	1	1	1	2	2	8	1	25
Platygastroidea	Platygastridae	0	0	3	24	1	1	2	18
	Scelionidae	10	89	11	466	12	76	11	312
Formicoidea	Formicidae	3	613	0	0	11	801	6	16
Evanioidea	Evaniidae	0	0	0	0	1	1	0	0
Unknown	Unidentified	1	3	3	6	1	4	3	5
Total Families		26		24		24		20	
TOTAL		100	1,544	68	840	89	1,044	65	523

Table 4: Diversity indices for sorghum and Johnsongrass with sugarcane aphid and checks by trap type.

Shannon-Wiener Diversity Index (Sorghum)					Simpson Diveristy Index (Sorghum)				
	Pan		Sticky			Pan		Sticky	
	With Aphids	Control	With Aphids	Control		With Aphids	Control	With Aphids	Control
7/31/2019	1.914	0.910	2.403	1.991	7/31/2019	0.749	1.000	0.884	0.810
8/17/2019	2.037	2.239	2.123	1.120	8/17/2019	0.780	0.871	0.732	0.612
9/2/2019	2.147	1.526	2.100	0.674	9/2/2019	0.794	0.751	0.836	0.475
9/9/2019	2.179	1.826	2.029	1.750	9/9/2019	0.767	0.772	0.739	0.810
9/16/2019	2.252	1.229	2.513	2.594	9/16/2019	0.841	0.628	0.875	0.931
9/27/2019	2.617	2.579	2.576	2.332	9/27/2019	0.840	0.953	0.868	0.890

Shannon-Wiener Diversity Index (Johnsongrass)					Simpson Diversity Index (Johnsongrass)				
	Pan		Sticky			Pan		Sticky	
	With Aphids	Control	With Aphids	Control		With Aphids	Control	With Aphids	Control
8/2/2019	2.509	1.300	1.888	1.851	8/2/2019	0.843	0.891	0.633	0.842
8/26/2019	2.259	1.600	2.082	1.709	8/26/2019	0.827	0.875	0.749	0.838
9/6/2019	2.177	N/A	2.376	N/A	9/6/2019	0.808	N/A	0.897	N/A
9/15/2019	2.039	N/A	2.695	N/A	9/15/2019	0.781	N/A	0.903	N/A
9/23/2019	2.387	N/A	2.587	N/A	9/23/2019	0.819	N/A	0.883	N/A

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CHAPTER IV

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

1. Host Plant Resistance

The sugarcane aphid causes major losses within sorghum crops which it infests. As it feeds, the plant's development is disrupted resulting in lower grain yields during harvest and overall economic losses for the grower (Bowling et al., 2016). The sugarcane aphid has the ability to reproduce asexually, which results in rapid population increase in just a few weeks. In order to protect crops, farmers may use chemical insecticides to control this species. While using chemical control is common practice, its use should be done in conjunction with other types of control because of economic costs of pesticides and their application and because of environmental costs to non-target and beneficial species.

This research examined grain sorghum varieties for innate resistance traits that can be used to sustain yield. Overall, 19 sorghum varieties were tested for resistance as against a known resistant variety. Three additional known susceptible varieties were also tested as positive controls. Of the 19 varieties tested, 9 showed moderate levels of resistance, while 2 showed very strong resistance toward SCA. While these results are promising, more research is needed to identify the types of resistance these plant display (Paudyal et al., 2019) and compare yields from mature plants by establishing field trials. The identification of resistance type would enable breeders to breed resistant plants with desired traits.

2. Aphid Honeydew Influence on Ants, Bees and Wasps

Honeydew is produced by true bugs, which feed on plant juices. The insects excrete honeydew as a waste product, and it contains sugars, and micronutrients (Fischer et al., 2005; Akbar et al., 2014). Aphids are able to reproduce quickly, and dense infestations will leave plants coated in honeydew. On sorghum, the sugarcane aphid, reproduces quickly and colonies can exceed thousands of individuals per leaf (Bowling et al., 2016). In these infestations, honeydew quickly builds up on the leaves below where the aphids are feeding.

Observations were made in sorghum infested with sugarcane aphid, and numerous species of bees and wasps were observed readily flying around the field feeding on the sugary excreta. Bees and wasps are important insects within agricultural settings as they pollinate, prey upon and utilize potential pest insects as hosts. I recorded increases in most types of Hymenoptera in fields with sugarcane aphid. The results from this experiment showed that the diversity of bees and wasps increased when the sugarcane aphid was present. More research is needed to see if the presence of sugarcane aphid could promote native insect populations in agricultural fields of sorghum. Increasing parasitoids and predators may also limit sugarcane aphid colonies improving yields.

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APPENDICES

Figure 1: This is a picture of the experimental flats and the infestation process



Figure 2: Hymenoptera Sorghum sites at the Cimarron Research Station

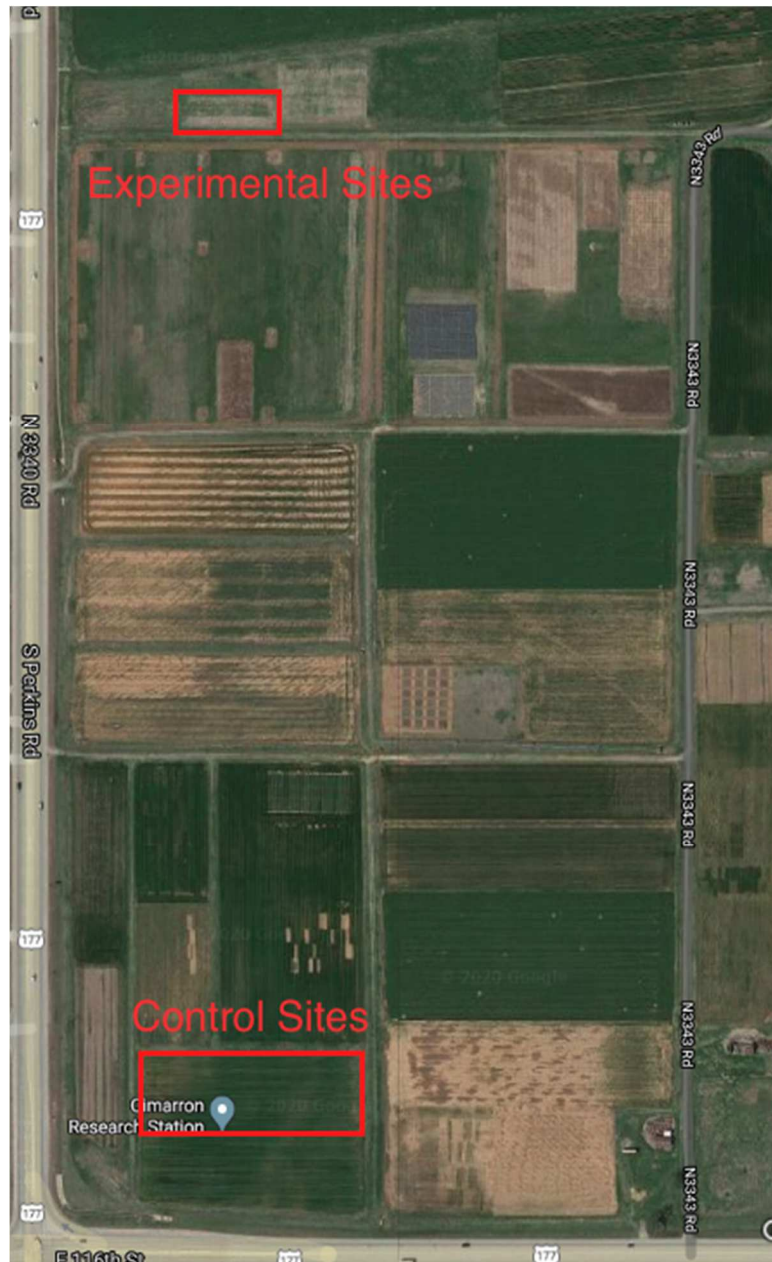


Figure 3: A few examples of the many different Hymenoptera observed feeding on the SCA honeydew



Figure 5: Sorghum Experimental sites. Left to right:P1-(1,2,3,4), P2-(1,2,3,4), P3-(1,2,3,4), P4-(1,2,3,4). 10m separated each site in a column, and 3.5m separated each column.



Figure 6: Each site's trap construction. Pan traps on the ground, and sticky card placed approximately at plant height.



Figure 7: Example of how the Hymenoptera were stored. Site, color pan trap, and date.



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