HOST PLANT RESISTANCE TO THE SUGARCANE APHID (HEMIPTERA: APHIDIDAE)

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2019

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ACKNOWLEDGEMENTS

I wish to express my gratitude to my major advisors, Drs. J. Scott Armstrong and Kristopher L. Giles, for their constructive guidance, constant help, patience, and encouragement throughout my program. I acknowledge their contributions without whose assistance this work would not have been completed.

I would like to thank Dr. George Opit for his constant support and guidance since I joined Oklahoma State University. I also express sincere thanks to Dr. Mark E. Payton for statistical and technical advice. The contribution and perspective of my advisory committee significantly helped me to improve the ideas and analyses.

Additionally, I would like to thank Dr. Karen R. Harris-Shultz and Dr. Hongliang Wang for providing me the practical knowledge of genetics and mentoring me in one of my research projects. Dr. Wyatt Hoback was a great help in teaching me skills about LI-COR and I am very grateful for his help.

This research could not have been completed without the assistance of the following people: Barbara Driskel, Hannah Baker, Ankur Limaje, and Misael Desouza. Moreover, I thank faculty, staff, and friends in the Department of Entomology and Plant Pathology for teaching and training me, and for their support during my graduate study. My special thanks go to my friends, Srijana and Pratishtha who supported me emotionally throughout my program.

I would also like to express the greatest of gratitude to the best family the world has to offer: my husband, Sarthak Khanal, my parents, Daya Ram and Gauri Paudyal, my in-laws, Surendra and Chandrakala Khanal, my siblings, Prashanta and Smriti for their love, support and encouragement throughout my doctorate and always being motivating forces for me in every steps of life.

Name: SULOCHANA PAUDYAL

Date of Degree: MAY, 2019

Title of Study: HOST PLANT RESISTANCE TO THE SUGARCANE APHID (HEMIPTERA: APHIDIDAE)

Major Field: ENTOMOLOGY

Abstract: The sugarcane aphid (*Melanaphis sacchari* (Zehnter) (Hemiptera: Aphididae)) has become a serious pest of sorghum (*Sorghum bicolor* (L.) Moench) in the United States since it was detected in 2013. Knowledge of the physiological response of sorghum to *M. sacchari* feeding will provide baseline information on a defined defense response and the resistance mechanisms of sorghum. This study documented the impact of *M. sacchari* feeding on resistant and susceptible genotypes through chlorophyll content, photosynthetic rate, stomatal conductance and carbon assimilation. Resistant genotypes that were infested with sugarcane aphids were able to compensate injury by either increasing or maintaining photosynthetic rate and stomatal conductance. Some resistant sorghum entries were able to tolerate the impacts of *M. sacchari* injury on photosynthetic integrity. Finding and advancing such germplasm has been a priority for sorghum breeders at the academic and industry levels. Twenty-three sorghum genotypes were selected and evaluated for resistance to *M. sacchari* by testing for tolerance, antibiosis and antixenosis. Free-choice and no-choice tests were conducted to explore the functional categories of resistance. Levels of resistance to *M. sacchari* were compared with the known resistant 'TX 2783' and susceptible 'KS 585' genotypes. Sorghum entries AG1201, AG1301, W844-E, and DKS 37-07 were identified as expressing tolerance, antibiosis, and antixenosis, while H13073 expressed antibiosis and GW1489 expressed both tolerance and antibiosis. Lastly, I examined the phenotypic behaviors (host suitability as measured through life table statistics) among the *M. sacchari* clones collected from different hosts and geographic locations. Aphid clones varied in performance among plant hosts. The survivorship and reproduction of the sugarcane collected aphid clone (SuSCA) was significantly higher when offered sugarcane (>85%) as compared to other hosts. In contrast, there was negligible survival and reproduction when sorghum collected (SoSCA) and Columbus grass collected (CoSCA) clones were offered sugarcane as host. This observation suggests that SuSCA and SoSCA are host-specific clones.

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CHAPTER I

INTRODUCTION

Plant-insect interactions are often dynamic, characterized by continual change, and based on 400 million years of co-evolution between herbivore and plant groups or pared species (Ehrlich and Raven 1964, Labandeira 2013, Bruce 2014). Relationships between insects and plants can be beneficial and/or harmful. Many plants provide nectar as food for many groups of insects, but large numbers of insect herbivores, including aphids, function primarily as a threat to plant growth and development. However, most plants have developed defensive strategies against aphids, and the resistance mechanisms include chemical and physical barriers (Howe and Schaller 2008, Jaouannet et al. 2014, Nalam et al. 2019). For agricultural crops, these barriers to aphid damage are regularly identified among germplasm sources and utilized in breeding programs to protect against plant damage and preserve yields (Hasan et al. 2015, Kumar et al. 2017, van Emdem 2017). Understanding crop-aphid interactions and outcomes requires knowledge of life cycles, feeding strategies, and ultimately the effect on plant physiology and growth.

Aphids are the major economic pests of cereal crops worldwide and they can directly damage plant tissues and reduce growth by feeding on phloem or indirectly affect plant growth by altering plant physiology and/or transmitting plant viruses (Dixon 2000, Quisenberry and Ni 2007, Van Emdem and Harrington 2007, Jaouannet et al. 2014).

Aphid life cycles are divided into a number of stages and often involve a sequence of morphs that specialize in feeding and reproduction, dispersal, or survival. For morphs specializing in feeding and reproduction, the timing of interactions with plant hosts influences plant nutrient conditions and ultimately yields (Fereres et al. 2017), and knowledge of these relationships allow for the development of reliable host plant resistance screening procedures to identify resistant germplasm sources.

The sugarcane aphid is a significant pest of sorghum worldwide (Hall 1987, Singh et al. 2004, McAllister et al. 2008, Chinnaraja and Viswanathan 2015, Bowling et al. 2016, Elliot et al. 2017). It invaded North America sorghum in 2013, although it has been reported in sugarcane, *Saccharum officinarum* (L.) since the 1980s (Mead 1978, White et al. 2001). Sugarcane aphids feed on the abaxial surface of sorghum leaves, which serve as a physical habitat and sink for their nutritional needs (Armstrong et al. 2015, Colares et al. 2015). Both nymphs and adults are efficient at sucking sap from the leaf tissue (Singh et al. 2004), and subsequently these aphids excrete large amounts of honeydew which often leads to growth of sooty mold (Bowling et al. 2016). Despite reports of significant yield losses associated with increasing infestations in sorghum (Bowling et al. 2016, Zapata et al. 2018, Brewer et al. 2019), little is actually known about the physiological effects of sugarcane aphid infestations.

Since the initial outbreak of the sugarcane aphid in sorghum during 2013, research has been conducted to identify integrated pest management strategies for aphid suppression. Chemical control strategies have been researched and implemented as seed treatments and justified foliar sprays following scouting (Villanueva et al. 2014, Jones et al. 2015, Bowling et al. 2016). However, chemical control measures are not always economically feasible in low-value sorghum and their compatibility with biological control must be carefully

considered (Singh et al. 2004, Bowling et al. 2016, Brewer et al. 2017). Various natural enemies of *M. sacchari* were reported in North America (Bowling et al. 2016), and recent work by Hewlett et al. (2019) suggest that ladybirds and lacewings have potential to suppress *M. sacchari* at low to medium aphid population densities (20 to 160 aphids per plant). However, the impact of other natural enemies and their contribution towards reducing *M. sacchari* populations below economic injury level should be considered.

Host plant resistance is one of the most effective and least disruptive IPM techniques as it helps to raise economic thresholds (ET's) and may delay or negate the need for insecticides. There are three classic categories of plant resistance: antibiosis, antixenosis, and tolerance (Painter 1951). Antibiosis can affect the biology of insects and decrease the chance of survival, whereas antixenosis reflects differential colonization or feeding preferences among plant genotypes. Tolerance is a characteristic that allows plants to resist attack by the pest (Painter 1951). Typically, however, pest populations can be influenced by multiple categories of resistance (Hill 2004, van Emden 2017).

Use of host plant resistance for the management of *M. sacchari* could be challenging because of evolution of sugarcane aphid genotypes (Nibouche et al. 2018). Genetic diversity has been examined worldwide for the sugarcane aphid and several multilocus genotypes have been identified (MLL-A, MLL-B, MLL-C, MLL-D, MLL-E, and MLL-F) (Nibouche et al. 2014, 2018). Recently, Nibouche et al. (2018) suggested that the MLL-F lineage is currently threatening the sorghum industry in the United States. Altogether, data from these studies indicate that there are likely host-associated genotypes of the SCA in the United States.

As previously stated, little is actually known about the effects of sugarcane aphid infestations on sorghum physiology and growth. Significant progress has been made towards identification of sorghum germplasm with resistance to sugarcane aphid, but knowledge of the mechanisms of resistance to the sugarcane aphid among resistant sources is needed for the breeding program.

Objectives

The objectives of this dissertation were to:

1. Investigate the physiological influence of sugarcane aphids (Hemiptera: Aphididae) on resistant and susceptible sorghum genotypes.
2. Categorize the mechanisms of resistance to the sugarcane aphid (Hemiptera: Aphididae) among sorghum genotype sources.
3. Identify phenotypic differentiation among sugarcane aphids from different plant hosts.

Significance of research

From these research objectives, I expect to quantify sugarcane aphid and sorghum interactions among selected germplasm in terms of resistance, physiology, and biology. Findings from these studies will be valuable for sorghum breeding programs focusing on sugarcane aphid resistance and are likely to result in more durable sorghum varieties.

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CHAPTER II

LITERATURE REVIEW

Sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the top five cereal crops produced in the world and is grown for grain, fiber, and fodder (Young and Teetes 1977, FAO 1995). It is the dietary staple food for more than 500 million people with adequate crude protein (8-12%) and high carbohydrate (65-80%) levels (Anjali et al. 2017). As it is devoid of gluten, it has been often recommended as a safe food for celiac patients (Ciacci et al. 2007, Kulamarva et al. 2009). There are various types of sorghum including, grain sorghum, forage sorghum, sweet sorghums and broomcorn (Hariprasanna and Rakshit 2016). Sorghum is a C4 plant and one of the most drought tolerant cereal crops that grows in semi-arid conditions (Taylor et. al. 2006). In the United States, it is considered the third most important cereal crop, after wheat and maize. Sorghum grain production in the United States has been estimated at 596, 480, 363.8, and 363.6 million bushels in 2015, 2016, 2017, and 2018, respectively (USDA-NASS, 2018). It is grown in more than 30 states, but Texas and Kansas are the largest producers (USDA-NASS, 2018).

Aphids and those attacking sorghum

Aphids (order Hemiptera, family Aphididae) are an economically important group of crop pests worldwide and able to cause severe damage by direct feeding on phloem or indirectly affecting plant immune systems by altering physiology and/or transmitting plant viruses (Quisenberry and Ni 2007). More than 4000 aphid species have been described, and some aphid species have host ranges limited to only a single plant family or frequently to multiple plant families (polyphagous) (Dixon 1998). Among aphid species, only about 100 species are suggested to have economic importance (Blackman and Eastop 2017).

Aphids are small, soft pear-like bodied insects with three pair of legs, a pair of antennae, and a pair of cornicles or siphunculi and vary in color from green, yellow, brown, pink, black to colorless (Dixon 1998). Their long antennae bear many sensilla important for chemoreception, gustation, and perception of leaf surface, and the pair of cornicles at the posterior end of the body secrete a defensive fluid containing alarm pheromone (E-β-farnesene) (Vandermoten et al. 2012). Aphids have piercing and sucking mouthparts, referred to ‘stylets’ and can penetrate mostly intracellular plant tissue and consume phloem sap (Dixon 1998).

Aphid life cycles are divided into a number of stages and often involve a sequence of morphs that specialize in feeding and reproduction, dispersal, or survival. (Williams and Dixon 2007). The life cycle and associated morphs affect the degree of damage in plants. Aphids can produce a female nymph without fertilization (known as parthenogenesis) and can continue doing this for many generations, however, under

certain conditions the winged or wingless male appear and sexual reproduction can occur (Dixon 1985). Such cycle is commonly known as cyclical parthenogenesis where clonal and sexual reproduction alternate within the annual life cycle (Dixon 1985, Simon et al. 2002). However, there are certain aphids which can reproduce exclusively by parthenogenesis (Simon et al. 2002, Williams and Dixon 2007). Aphid parthenogenesis is associated with viviparity and the telescoping of generations, where a granddaughter of female aphid is already developing within the daughter inside her (Simon et al. 2002, Miura et al. 2003). For all species, winged parthenogenetic morph (alate) reproduction rate is lower than the wingless morph (apterous) and the reason why they have great reproductive potential than other sexually reproducing animals (Powell et al. 2006, Blackman and Eastop 2017).

There are several species of aphids that attack sorghum, but the most common species are: *Schizaphis graminum* (greenbug*), Rhopalosiphum maidis* (corn leaf aphid), *Sipha flava* (yellow sugarcane aphid), and *Melanaphis sacchari* (sugarcane aphid) (Michaud 2017). These aphid species (adult and nymph stages) can be easily distinguished by using 10 power magnification (Bowling et al. 2016a). It is often more difficult to identify different damage symptoms and physiological injury caused by different aphid species. The greenbug is pale green in color with a dark green mid-dorsal stripe and dark tarsi, antennae, and cornicle tips (Royer et al. 2015). Plant damage by greenbug, which is believed to be caused by toxic saliva, is most easily identified and results in subsequent formation of red necrotic spots at the feeding sites (Girma et al. 1998, Royer et al. 2015). The corn leaf aphid is a blue-green in color with black legs, (van Emdem, 2007), and is known to suck sap from upper wilt (Li et al. 2008). The

yellow sugarcane aphid is oval bright yellow in color with numerous long bristle-like hairs on the body. The sugarcane aphid can be distinguished by a grey to tan yellow body color and the presence of distinct dark cornicles, tarsi, and antennae but feeding injury on sorghum appears similar to corn leaf and yellow sugarcane aphids (Bowling et al. 2016a).

The Sugarcane Aphid:

The sugarcane aphid belongs to the order Hemiptera, Suborder Sternorrhyncha, superfamily Aphidoidea, and family Aphididae. The pest status of the sugarcane aphid in sorghum has been well documented from different countries, India, China, South Africa, Japan, and most recently from the United States (Singh et al. 2004, Armstrong et al. 2013, Bowling et al. 2016). In the United States, the sugarcane aphid was first reported in 1877 in Florida (Mead 1978, Hall 1977) and in 1999 in Louisiana on sugarcane (*Saccharum officinarum* L.) (White et al. 2001). An outbreak of the sugarcane aphid in sorghum was first reported near Beaumont, Texas in 2013 (Brewer et al. 2017). By the end of 2013, it was reported from 38 counties from four different states including, Texas, Louisiana, Mississippi and Oklahoma (Knutson et al. 2016, Bowling et al. 2016a) and has subsequently expanded its geographic range to 20 different states (Long et al. 2018). In infested regions of the U.S., it is now common to encounter large populations of the aphid in most fields annually.

Among aphid, the sugarcane aphid sucks large amounts of sap from plant tissue, producing a tremendous amount of honeydew which allows for extensive colonization of sooty mold on plants (Singh et al. 2004, Bowling et al. 2016a). The sugarcane aphid feeds on sorghum during spring and summer growing months and can survive during the

winter on grasses including Johnsongrass, Columbus grass in Southern Texas (Armstrong et al. 2015, Bowling et al. 2016a).

The sugarcane aphid is generally found as parthenogenetic and anholocyclic which means alate (winged) and apterous (wingless) females are produced by asexual means. The sexual form has not been reported in the U.S., however, it has been reported in India (David and Sandhu 1976). The winged morph of the sugarcane aphid can also be distinguished by the black markings along the dorsal sclerites and hardened structures at the base of the wings (Bowling et al. 2016a). Winged sugarcane aphids are able to dispersed from short to long distances, rapidly colonize new areas, and following rapid population increases cause damage to plants by feeding, transmitting pathogens, and/or promoting colonization of sooty mold (Bowling et al. 2016a). Nymphs pass through four different stages, which has been observed to take 4 to 12 days (Chang et al. 1982). The reproductive potential of the aphid has been reported between 34 to 96 nymphs per female and longevity ranges from 10-37 days depending on environmental conditions (Chang et al. 1982, Singh et al. 2004).

Host Plant Range

Sugarcane aphids are reported as a minor pest of several crops and have 14 known suitable host plants worldwide which include *Cynodon dactylon* (L.), *Miscanthus sinensis* (L.), *Oryza sativa* (L.), *Panicum colonum*, *Panicum maximum*, *Paspalum sanguinale*, *Pennisetum* sp., *Saccharum officinarum*, *Setaria italic* (L.), *S. bicolor*, *Sorghum halepense* (L.) Pers. (Johnsongrass), *S. verticilliflorum* (Steud.), and *Zea mays* (L.) (Singh et al. 2004). To date, the predominant biotype in the U.S. has a host range limited

to *S. bicolor*, *S. halepense*, *Saccharum officinarum*, Sudan grass (*Sorghum*× *drummondii*), and Columbus grass (*Sorghum* × *almum*) (White et al. 2001, Armstrong et al. 2015, Medina et al. 2016).

Plant injury and economic damage

Sugarcane aphids suck large amount of sap from plants, and heavy infestations may lead to purpling of leaves and reduced crop growth and yield components particularly grain quality which ultimately effects the brewing and milling net profits (van den Berg et al. 2003). It is also known as a massive honeydew producer, which is comprised of amino acids and sugars, makes leaves shiny and sticky and may hinder harvesting by choking the combines (Villanueva et al. 2014). Honeydew fosters the growth of black sooty molds, which can cover the leaves or entire plant including the panicle, that often causes yellowing and eventually death of the plant tissue (Bowling et al. 2016a). Aphids are known to remove photo assimilates and sugarcane aphid-infested plants have decreases leaf chlorophyll content which causes a reduction in plant height (Limaje et al. 2017, Backoulou et al. 2018). Such stressed plants have uneven or lack of head emergence and poor grain set which can be more severe if sugarcane aphids transmit sugarcane yellow leaf virus to plants (Rott et al. 2008).

The sugarcane aphid can infest sorghum from early to mature stage, however, significant economic losses have primarily been reported when infestations occur at the flowering or grain filling stages (Raetano and Nakano 1994). Sorghum yield losses have been reported from different parts of the world ranging from minor to severe (Bowling et al. 2016a) and are estimated at 100-400 lb/acre yield loss when aphid reach 50-500 per

leaf during the pre-flowering stage. Survey results in the Rio Grande Valley, Texas estimated loss of $64.53/ac between 2014 and 2015 due to sugarcane aphid infestations driven primarily by increased production costs as well as reduced sorghum yields (Zapata et al. 2018).

Genetic diversity of the sugarcane aphid

Genetic diversity of the sugarcane aphid has recently been examined worldwide and in the Americas. A recent study reported six multilocus lineages including, MLL-A, MLL-B, MLL-C, MLL-D, MLL-E, and MLL-F and suggested MLL-F is the lineage which is currently threatening the sorghum industry in the United States since 2013 (Ninouche et al. 2015, 2018). MLL-F is considered an invader to the Americas from Africa or Asia (Nibouche et al. 2018), and is genetically different than populations collected on sugarcane and Johnsongrass in 2007 from Louisiana and Hawaii (Nibouche et al. 2015). For sugarcane aphid samples collected after 2013 in the continental U.S., sugarcane aphids that were MLL-D were found only on sugarcane, but sugarcane aphid samples that were MLL-F were found on sugarcane, sorghum, and Johnsongrass (Nibouche et al. 2018). Most likely, use of sorghum as a host is not because of a host switch as speculated previously, but because of the introduction of new genotypes probably from Asia or Afria (Nibouche et al. 2018).

Management strategies:

Integrated pest management (IPM) is described as a comprehensive pest management approach which focuses on the suppression of pest population to tolerable levels using one or more control tactics, while minimizing applications of disruptive chemical pesticides (Stenberg 2017), in a way that is both environmentally friendly and economically favorable (Ehler 2006). Knowledge of pest life cycles, host preferences, population dynamics, interactions with other species allow for the development of effective IPM strategies. Despite the recent importance of the sugarcane aphid in sorghum, very little information is currently available on sustainable best management strategies. Since this pest is relatively new to sorghum in the U.S., a thorough understanding of the pest status, interactions with plants, natural enemy interactions, production and environmental influences are still lacking. Increasing attention has been paid to host plant resistance (Singh et al. 2004, Armstrong et al. 2015, 2017, Mbulwe et al. 2016, Limaje et al. 2017), proper insecticide as a management plans (Etheridge et al. 2018), and incorporating the impact of biological control (Bowling et al. 2016, Hewlett et al. 2019).

Scouting and Early detection

Early detection of the sugarcane aphid and rapid management decisions allow producers to decrease economic losses (Bowling et al. 2016a, McCornack et al. 2017). Bowling et al. (2016a and 2016b) developed an efficient weekly scouting protocol. Aphid populations can be estimated based on visual observations of leaves below the flag leaf (Bowling et al. 2016b).

Biological Control

Several insect predators and parasitoids have been reported to reduce populations of aphids. In a review paper by Singh et al. (2004), natural enemies of *M. sacchari* include 1 pathogen, 6 parasitoids, and 38 predators. The arrival of *M. sacchari* in North America triggered interest in understanding the roles of existing natural enemies and a survey conducted in Texas reported several potentially important natural enemies of *M. sacchari*: nine lady bird species (Coleoptera: Coccinellidae), brown lacewing (Neuroptera: Hemerobiidae), five species of green lacewing (Neuroptera: Chrysopidae), hoverflies (Diptera: Syrphidae), minute pirate bug (Hemiptera: Anthocoridae), the parasitoid, *Aphelinus sp*. (Hymenoptera: Aphelinidae) and a hyperparasitoid, *Lysiphlebus testsceipes* (Cresson) (Hymenoptera: Braconidae) (Bowling et al. 2016a). Hewlett et al. (2019) suggested that ladybirds and lacewings have the potential to suppress *M. sacchari* at low to medium aphid population densities (20 to 160 aphids per plant). Hall (1987) found that the efficacy of the fungal pathogen (*Verticillium lecanii*) was highest at low population levels. Additionally, there is a recent report about the natural presence of entomopathogenic fungi (*Lecanicillium longisporum*, *Beauveria bassiana*, and *Isaria javanica*) infecting *M. sacchari* in Mexico but it is not known if aphids are reliably suppressed by these pathogens (Zambrano-Gutierrez et al. 2019). To date, *M. sacchari* has not been observed to be consistently reduced below economic injury levels by naturally occurring natural enemies (Hall 1987, Bowling et all 2016a, Zambrano-Gutierrez et al. 2019, Hewlett et al. 2019).

Chemical Control

Insecticidal spray decisions depend on the number of aphids per plant and seed treatment with a registered insecticide has been the first approach to reduce or negate the need for later foliar insecticide sprays (Bowling et al. 2016a). The curative recommended insecticides that are currently available for the management of *M. sacchari* are limited to two foliar products: Sivanto® 200SL (flupyradifurone) and Transform® WG (sulfoxaflor) (Bowling et al. 2016, Etheridge et al. 2018). Sulfoxaflor (Transform™ 50WG, Dow AgroSciences, Indianapolis, IN) is under Section 18 emergency exemption and flupyradifurone (SivantoTM 200SL Bayer CropScience, Leverkusen, Germany) was granted a Section 3 label (Bowling et al. 2016a). Zarrabi et al. (2018) tested these insecticides against *M. sacchari* and found that both reduce populations better than untreated control. Growers are concerned that reliance on such a limited chemical control strategy might lead to insecticide resistance and loss of effective curative controls (Whalon et al. 2008).

Host plant resistance

Host plant resistance is defined by Painter (1951) as the relative amount of heritable qualities possessed by plants which influences the ultimate degree of damage done by insects. Crop varieties that are resistant will be less damaged or infested by insects compared to others under comparable environmental conditions. Developing resistant varieties is highly valued in integrated pest management programs because it is an environment-friendly approach and compatible with other integrated pest management methods (Wilde 2002). Evaluation and deployment of resistance varieties against the

sugarcane aphid remains both a short and long-term priority and this sustainable tactic is considered a cornerstone of future management programs.

The relationship between plants and insects depends on the mechanisms of resistance. There are three mechanisms of resistance to insects defined by Painter (1951), antibiosis, non-preference, and tolerance, later non-preference is renamed as ‘antixenosis’. Morphological (plant trichrome, surface waxes, tissue thickness) or biochemical (repellants, antifeeding constituents, and deterrents) attributes of plants that alter insect behavior, especially the selection of plants and colonization is referred to as ‘antixenosis' (Smith 2005). Antibiosis is described as the adverse effect of host plant on the insect reproduction, development and survival, whereas tolerance is defined as the ability of plants to withstand the injury caused by insect feeding. Tolerant plants are believed to be most useful in sustainable long-term IPM programs because there is no selection pressure on the insect population (Smith 2005).

Plant resistance to aphids has been investigated either by screening tests (Armstrong et al. 2017) or feeding behavior tests by using electrical penetration graph (EPG) techniques (Todd et al. 2015). In both screening or EPG tests, seedlings are often used to investigate resistance mechanisms because seedlings are easier to handle and evaluate (Teetes et al. 1995). Sources of resistant sorghum to sugarcane aphids have been investigated in different countries since the early 2000’s (Singh et al. 2004). In the United States, Armstrong et al. (2015) identified eight resistant genotypes to sugarcane aphid and two lines B11055 and R13219 with higher degree of tolerance, antixenosis, and antibiosis (Armstrong et al. 2017). Bowling et al. (2016a) and Mbulwe et al. (2016) reported that several Texas A&M sorghum lines and hybrids including Tx2783, Tx3408, Tx3409,

B11070, AB11055-WF1-CS1/RTx436, and AB11055-WF1-CS1/RTx437 were resistant to sugarcane aphid feeding. Further research by Limaje et al. (2017) identified two parental lines, R. 11143 and R. 11259, with significant levels of tolerance and antibiosis to sugarcane aphid. Identifying agronomically acceptable sorghum entries with durable resistance to sugarcane aphids and identifying the mechanisms of resistance are important initial goals for this pest.

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CHAPTER III

THE PHYSIOLOGICAL INFLUENCE OF SUGARCANE APHIDS (HEMIPTERA: APHIDIDAE) ON RESISTANT AND SUSCEPTIBLE SORGHUM GENOTYPES

Abstract. Knowledge of physiological responses of sorghum, (*Sorghum bicolor* (L.) Moench), to sugarcane aphid (SCA), *Melanaphis sacchari* (Zehnter) feeding will provide baseline information on defense responses and resistance mechanisms. This study documented the impact of SCA feeding on seven sorghum genotypes through measures of chlorophyll content, photosynthetic rate, stomatal conductance, and carbon assimilation at 14-d post-infestation. Carbon assimilation (A/Ci) curves were recorded at 3, 6, 9, and 15 d after aphid introduction to understand the pattern of physiological response of resistant and susceptible sorghum over time. Chlorophyll loss of resistant genotypes was significantly lower (≤10% loss) than the susceptible indicating tolerance. Most resistant genotypes compensated injury by either increasing or maintaining photosynthetic rate and stomatal conductance in the infested plants compared to checks. A/Ci curves over time showed that infested resistant plants had delays in photosynthetic decrease, whereas susceptible plants displayed accelerated photosynthetic senescence. This research also investigated the influence of aphid density (0, 50, 100, 200) on photosynthetic rate of 28-d-old resistant and susceptible sorghum measured at 72-h post-infestation. Although, there were no visual symptoms in susceptible genotypes, photosynthetic rates were impaired when infested with ≥ 100 SCA. However, resistant plants were able to compensate when infested with ≤ 100 aphids. Differences between physiological responses of infested susceptible and resistant genotypes imply that resistant sorghum plants can tolerate some impacts of SCA injury and maintain photosynthetic integrity. Future studies should address physiological responses at later sorghum plant growth stages.

Keywords: *M. sacchari*, Photosynthetic rate, Sorghum, Chlorophyll

Introduction

The sugarcane aphid (SCA) (*Melanaphis sacchari* (Zehnter)) is a pest of sugarcane and sorghum in India, China, South Africa, Japan, and most recently the United States (Singh et al. 2004, Bowling et al. 2016). Although first reported in 1877 in Florida (Mead 1978, Hall 1987) and in 1999 in Louisiana on sugarcane (White et al. 2001), the aphid has caused considerable yield loss in sorghum crops in the United States since its association with sorghum in 2013 in Beaumont, Texas (Armstrong et al. 2015, Elliot et al. 2017, Brewer et al. 2017). The sugarcane aphid is typically found on the underside of sorghum leaves, where it feeds and excretes honeydew while accumulating amino acids, and carbohydrates (Bowling et al. 2016). It is believed that SCA pierce their host to remove sap from the xylem tissue of leaves (Singh et al. 2004), yet little is known about how *M. sacchari* exploit this tissue.

Plant resistance is viewed as cornerstone approach for the management of SCA, and a number of resistant sorghum genotypes have already been identified (Armstrong et al. 2015, Bowling et al. 2016, Limaje et al. 2017, Paudyal et al. unpublished data). In the process of further developing resistant sorghum to SCA, it is important to understand physiological mechanisms of resistance.

Aphids, in general have the potential to alter plant physiological processes (photosynthesis, stomatal conductance, respiration, chlorophyll content), which ultimately affects plant growth, development, and yield (Ryan et al. 1987, Meyer and Whitlow 1992, Larson 1998, Haile et al. 1999, Macedo, et al. 2003a, Diaz-Montano et al. 2007, Pierson et al. 2011). Feeding by SCA could inhibit the processes of photosynthesis

as well as reduce chlorophyll content of sorghum leaves. Understanding physiological alterations that result from SCA feeding is an important step for the development of accurate economic injury levels for different genotypes (Peterson and Higley 2001, Gordy et al. 2019).

Several studies have reported the impact of aphid feeding on plant physiological response and found reduced chlorophyll content of the plants (Miller et al. 1994, Deol et al. 2001, Diaz-Montano et al. 2007, Limaje et al. 2017). However, the reduction in chlorophyll may not indicate a net reduction in photosynthetic rate (Nagaraj et al. 2002). Aphids have also been described as having the potential to directly reduce photosynthesis either by preventing normal nutrient flow or by blocking stomatal opening and thus limiting CO_2 uptake because of honeydew accumulation on leaves (Haile et al. 1999, Frazen et al. 2007). However, some plants have the ability to compensate photosynthetic rates in response to insect attack (Retuerto et al. 2004, Gutsche et al. 2009).

The effects of aphid feeding injury on plant physiology has been studied in several crops (Haile et al. 1999, Haile and Higley 2003, Macedo et al. 2003a and b, Frazen et al. 2007, Pierson et al. 2011). Haile et al. (1999) reported an inverse relationship between the number of Russian wheat aphids (*Diuraphis noxia* (Mordvilko) and the photosynthesis rate on wheat. Tolerant wheat plants were able to recover photosynthetic capacity when aphids were removed after 7 d, but susceptible plants were unable to recover. Macedo et al. (2003a) reported similar results for wheat under continuous light, however under continuous dark for 72-h, there was no change in photosynthesis caused by Russian wheat aphids. A study conducted by Frazen et al. (2007) demonstrated that tolerant wheat had similar photosynthetic rates when compared

with the control, and an antibiotic resistant cultivar had delayed photosynthetic senescence. However, Gomez et al. (2006) found no alteration in the photosynthetic rate in cotton when infested with cotton aphids (*Aphis gossypii* G.) for 9 days.

To date, no studies report relationship between SCA injury and sorghum leaf photosynthesis and gas-exchange response. This knowledge of how SCA affects sorghum physiology especially among resistant and susceptible lines may help to explain physiological mechanisms underlying tolerance. The purpose of these experiments was to gain insight into the physiological responses of selected resistant and susceptible sorghum genotypes to SCA feeding. The specific objective was to describe the effects of SCA feeding on photosynthetic rates of resistant and susceptible over different time period.

Materials and Methods

Insects and Plant Materials The SCA used in this study were collected from grain sorghum at Matagora County, TX, in August of 2013 and maintained on susceptible genotype 'RTx7000' in 4.4-L pots fitted with 45-cm tall × 16-cm diameter cylinders of Lexan^{TM} (SABIC Polymershapes, Tulsa, OK) cages which were ventilated with organdy tops to prevent aphids from escaping. The SCA colony on RTx7000 was kept in a greenhouse at 21-31^{o}C and provided with two T-8 fluorescent lights.

Susceptible genotypes KS 585 and WSH 117 and resistant genotypes, AG1201, AG1203, AG1301, TX2783, and DKS 37-07 (Paudyal et al. Unpublished data) were used for assessing the response of photosynthetic capacity of plants to aphid attack. These genotypes were planted in Cone-tainers^{TM} (model SC10; S7S Greenhouse Supply,

Tangent, OR) containing three layers of media, potting soil, fitting clay, and sand (bottom to top respectively). Each Cone-tainer was fitted with an 8-cm-diameter Lexan sleeve, 45 cm in height and ventilated with organdy cloth. Two seeds of each genotype were planted to a depth of approximately 2 cm. Seedlings of each genotype were grown in a greenhouse under T-6 fluorescent lighting and 25 ± 3ºC and thinned to one per Cone-tainer one week after planting. Fourteen days after planting (four leaf stage), sorghum seedlings were infested with 20 sugarcane aphids. Uninfested plants (check) were caged like infested plants. Physiological responses were recorded at 15 d after aphid introduction in the seven genotypes. SCA were removed from plants before measuring the physiological response. There were five replications and two treatments (infested and check) for each genotype (7 genotypes * 5 replications * 2 treatments = 70 plants).

Chlorophyll Concentration A chlorophyll meter (model SPAD-502, Minolta Camera Co., Osaka, Japan) was used for the measurement of chlorophyll level in the seedlings. The meter is a hand held device that absorbs light at wavelengths between 430 and 750 nm when passed over a leaf and allows for estimates the chlorophyll content (Wood et al. 1992). Three readings from each seedling were measured for infested and check seedlings. These readings were averaged and a SPAD chlorophyll-index was calculated using mean SPAD based on formula: (C-T)/C (Deol et al. 1997), where C is SPAD measurement from the check and T is the SPAD measurement from infested plants.

Gas exchange response Photosynthesis responses were recorded from check and infested plants for all seven genotypes using a portable photosynthesis system (model LI-6400, LI-COR, Lincoln, NE). Similar to the methods by Gutsche et al. (2009) and Frazen et al. (2007), measurements were taken outdoors after plants were acclimatized for >1 h.

The photosynthetic parameters were measured from a 6-cm^2 area. If the width of the leaf was less than the area that fit into the LI-COR chamber, then two leaves were selected that fit in IRGA chamber. Gas-exchange parameters, including net photosynthetic rate (Pn), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were recorded at 15 d after sugarcane aphid infestation at 1200 umol photons $m^{-2}s^{-1}$ light intensity and a reference CO_2 of 400 ppm generated from a 12-g CO_2 cylinder connected to the meter. Stomatal conductance is the rate at which CO_2 entering or water vapor exiting through the stomata and internal CO_2 is the concentration of carbon dioxide inside the leaf (Meyer and Whitlow 1992). A second experiment was conducted to investigate the effect of SCA densities (0, 50, 100, 200) on photosynthetic rate of 28-d-old resistant (DKS 37-07) and susceptible (KS585) sorghum plants after 3-d of infestation. There were five replications for each density and genotype. Similar to above experiment, aphids were removed from plants before photosynthetic measurements.

To further understand the impact of SCA infestation on photosynthetic capacity of resistant and susceptible plants, CO_2 response curves (A/Ci) were examined at 3, 6, 9, and 14 d after infestation. Known resistant (DKS-3707) and susceptible genotypes (KS585) were selected for the measurement. The susceptible genotype, WSH117 was not selected due to limited green leaf tissue. Rates were measured at CO_2 concentrations ranging from 50 to 1000 ppm (sequence of 400, 200, 100, 50, 400, 400, 600, 800, 1000, 400 ppm) and at 1200 μmol photon $m^{-2}s^{-1}$ light intensity. A/Ci response curves were determined by the automated programs of LI-6400. After generating A/Ci curves, the parameters Vmax and Km were calculated from Lineweaver-Burk (equation 1) and its linear transformation (equation 2) (Burnell and Hatch 1988, Alla and Hassan 2012).

$$1/v = (1/[S]) (Km/Vmax) + (1/Vmax)$$

$$v= (Vmax[S])/ (Km + [S])$$

Where,

[S] = Substrate concentrations

Vmax = Maximum velocity (maximum rate of PEP carboxylation)

Km = Michaelis-Menten constant

Data analysis Mixed model analysis (PROC MIXED, SAS Institute 2009) was conducted for each measurement to examine differences in chlorophyll levels, net photosynthetic rate, and stomatal conductance. Parameters were analyzed by a 7(sorghum entries) * 2(SCA infested and uninfested) ANOVA with five replications. When appropriate, the means were separated according to protected Fisher-type pairwise comparisons. Similar analysis was conducted for analyzing the effect of aphid density on sorghum plants (4 densities and 2 genotypes). Statistical significance was assumed when $P<0.05$. A/Ci curves were established with a non-linear regression model (exponential rise to maximum with 3 parameter estimation) in Sigma Plot ® (version 10.0) software (Sysstat Software Inc.) at 3, 6, 9, and 14 d after infestation.

Results and Discussion

Chlorophyll loss from SCA feeding varied among sorghum entries (Table 1). The results clearly demonstrate that SCA infestation can have a negative impact on the chlorophyll of sorghum leaves (>60% of loss), however resistant plants had less

chlorophyll loss (≤10% of loss). Maintaining a relatively high chlorophyll content despite infestation is considered a good indicator of plant tolerance to herbivores (Lage et al. 2003).

Measurement of photosynthetic rate and stomatal conductance was significantly different among the genotypes and between treatments (infested and control) (Table 1). However, there was no significant difference in the intercellular CO_2 concentration (Ci) among genotypes or between treatments. The Ci values ranged from 112 to 208.6 ppm. Stomatal conductance (gs) was reduced significantly in two genotypes (KS585 and WSH 117), however all other genotypes (DKS 37-07, AG 1203, AG 1201, AG 1301, and TX 2783) showed similar conductance. The reduction of conductance in KS585 and WSH 117 suggest stomatal interference contributes to decreased photosynthetic rates (Meyer and Whitlow 1992). Photosynthetic rates of the check plants were not significantly different, whereas rates for injured plants were significantly reduced; DKS 37-07 had the highest photosynthetic rate, while KS 585 the lowest (Table 1).

Variations in the photosynthetic rate might suggest different physiological mechanisms for resistance (Pierson et al. 2011). Furthermore, SCA injury significantly reduced the photosynthetic rate of AG1301, but had no effect on TX 2783 and AG 1201. In this study, we cannot definitively answer why there was a reduction of photosynthetic rate in AG1301, despite its known resistance (Paudyal et al. unpublished data). Significantly higher photosynthetic rates were observed in two resistant entries, DKS 37-07 and AG 1203 infested with SCA than in checks. This might indicate that the plants increase their photosynthetic rate in order to compensate for the feeding injury caused by SCA (Pierson et al. 2011).

In order to understand more about the difference in the physiology for the resistance and susceptible plants, A/Ci curves at different time periods were developed and visual symptoms of the SCA were observed over time. Photosynthetic rates in A/Ci curves were affected by SCA infestation and over the course of the experiment, SCA infestation had different effects on resistant and susceptible sorghum plants. When a plant senesces, reduction in photosynthetic capacity is often observed (Gutsche et al. 2009). Results from this study suggest that resistant plants infested with SCA maintained or compensated for aphid injury by altering senescence pathways, whereas susceptible plants seemed to have an accelerated pathway. A/Ci curves were similar between infested and control sorghum plants as well as resistant and susceptible genotypes at 3 d after infestation (Fig. 1), indicating that SCA feeding did not alter carbon fixation. Assimilation rate was significantly reduced in susceptible plants at 6 d after infestation. Similar shallow A/Ci slopes were observed at 9 and 14 d after SCA infestations in susceptible plants which might indicate lower RuBP regeneration as suggested by Farquer et al. (1980) and Sharkey (1985). The steep slopes of resistant sorghum throughout the experiment might suggest efficient RuBP regeneration similar to the results reported by Frazen et al. (2007) and Gutsche et al. (2009). However, Frazen et al. (2007) and Gutsche et al. (2009) reported effects of Russian wheat aphid on wheat which is C3 plant, and it has been described that photosynthetic reactions differ between C3 and C4 plants (Yamori et al. 2014, Nagaraj et al. 2002, Yin and Struik 2009).

The relationship between A and Ci was modelled using the Lineweaver model to derive important PEPC kinetic parameters (maximum carboxylation rate (Vmax) and Michaelis-Menten Constant for CO_2 (Kp)). These kinetic parameters define the enzyme-

catalyzed reaction rate as substrate concentration varies. Decreases in the value of Vmax indicates a decrease in the enzyme active site participation in the reaction because of aphid feeding (Alla and Hasan 2012). Additionally, the increase in Km reflects a greater interference of the aphid on structural integrity of enzymes. Decrease in Vmax and increase in Km in the susceptible host plants suggest that sugarcane aphids are able to interfere with the enzyme activity.

Sorghum plants did not show any visible injury from SCA infestation (20 aphids) for the first six d after infestation. After six d, susceptible sorghum plants were observed to wilt, become chlorotic and eventually senesce. The results of this study showed the symptoms of SCA feeding was a result of chlorophyll loss, reduction in photosynthesis and stomatal closure. Future studies should be conducted to detect whether decreases in physiological parameters from infestations at early stages of sorghum development would decrease above-ground biomass, that ultimately causes declines in grain yield.

This research provides the first report on the physiological response to SCA feeding and demonstrated that sorghum resistant plants appeared to be able to compensate for injury caused by SCA feeding by maintaining/increasing photosynthetic capacity. These findings support previous research with other aphid species (Retuerto et al. 2004, Heng-Moss et al. 2006, Frazen et al. 2007, Gutsche et al. 2009) on C3 plants. Knowledge of the physiological alterations occurring in sorghum leaves infested by SCA may be leveraged into the development of new resistant cultivars. Future study should be conducted to address physiological responses of mature plants since the impact of SCA on mature sorghum (vegetative stage, reproductive stage, for example) may be different.

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Table 1. Effect of *M. sacchari* on gas-exchange responses and chlorophyll of sorghum entries at 14 d after infestation with 20 aphids.

Entries	Photosynthetic rate		Stomatal Conductance		Chlorophyll loss (%)
	Infested	Control	Infested	Control	(Control-Infested)/Control
DKS 37-07	15.87 ± 1.04 c	12.08 ± 0.29 b	0.12 ± 0.01 b	0.11 ± 0.01 b	6.33 ± 1.73 a
AG 1203	15.57 ± 0.09 c	11.82 ± 1.45 b	0.11 ± 0.02 b	0.13 ± 0.01 b	6.07 ± 0.81 a
Ag1201	13.75 ± 0.64 bc	13.91 ± 0.61 bc	0.12 ± 0.01 b	0.11 ± 0.01 b	5.34 ± 1.09 a
TX2783	11.84 ± 0.54 b	11.23 ± 1.08 b	0.11 ± 0.02 b	0.12 ± 0.01 b	9.33 ± 2.23 a
AG 1301	5.92 ± 0.45 a	13.56 ± 1.88 bc	0.08 ± 0.01 b	0.10 ± 0.01 b	10.82 ± 1.21 a
KS 585	4.71 ± 1.7 a	12.02 ± 2.34 bc	0.04 ± 0.02 a	0.11 ± 0.00 b	68.48 ± 7.89 c
WSH117	3.68 ± 2.26 a	11.92 ± 0.38 b	0.02 ± 0.01 a	0.12 ± 0.02 b	24. 87 ± 5.29 b
	Entry: df= 6, 52, F=10.42 P<0.001		Entry: df= 6, 52, F=7.14 P<0.001		df= 6, 24; F=66.63; P<0.001
	Treatment (Infested vs Control): df= 1, 52 F=33.06 P<0.001		Treatment (Infested vs Control): df= 1, 52 F=18.17 P<0.001		
	Entry* Treatment: df= 6, 52, F= 8.96, P<0.001		Entry* Treatment: df= 6, 52, F= 5.26, P<0.001		

Means for Photosynthetic rate, Stomatal Conductance, and Chlorophyll loss (%) followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD

Table 2: Parameters (Km and Vmax) estimated at 3, 6, 9, and 14 d after infestation in check and infested treatments in susceptible (KS 585) and resistant (DKS-37-07) genotypes.

Days after infestation	Entry	Km (Michaelis Constant)			Vmax (Maximum rate of reaction)		
		Check	Infested	p-value	Check	Infested	p-value
3	Susceptible	6.21 ± 0.74	5.16 ± 0.08	0.83	22.85 ± 1.88	19.51 ± 2.89	0.14
	Resistant	5.50 ± 1.38	5.87 ± 0.96	0.47	18.18 ± 2.41	21.83 ± 1.86	0.21
6	Susceptible	5.17 ± 0.98	6.46 ± 0.71	0.83	22.84 ± 0.15	13.63 ± 0.37	0.0003
	Resistant	6.38 ± 1.14	7.64 ± 0.91	0.56	19.68 ± 2.27	22.02 ± 0.72	0.30
9	Susceptible	6.16 ± 0.13	10.41 ± 3.38	0.54	19.65 ± 0.56	4.74 ± 0.78	<0.0001
	Resistant	6.35 ± 1.18	6.73 ± 0.45	0.46	18.51 ± 2.15	26.57 ± 0.34	0.0003
14	Susceptible	5.78 ± 0.19	18.85 ± 1.88	0.02	17.93 ± 2.06	7.37 ± 0.62	<0.0001
	Resistant	6.68 ± 0.49	7.69 ± 0.34	<0.0001	19.01 ± 2.26	24.44 ± 0.28	0.02

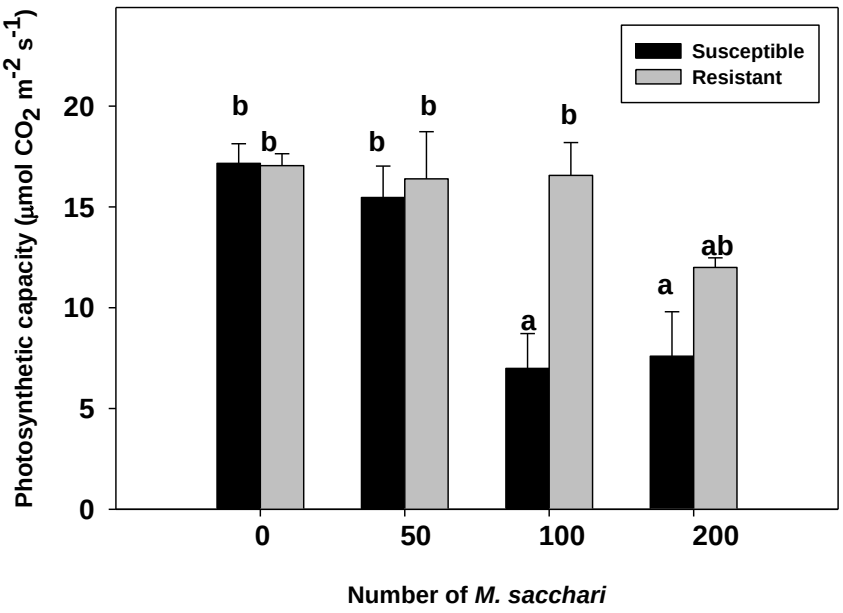

Figure 1. Photosynthetic capacity (μmol CO2 m^{-2} s^{-1}) of 28-d old resistant (DKS 37-07) and suceptible(KS 585) sorghum gneotypes at four densities of *M. sacchari* (0, 50, 100, 200) at 3-d after infestation. Means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD.

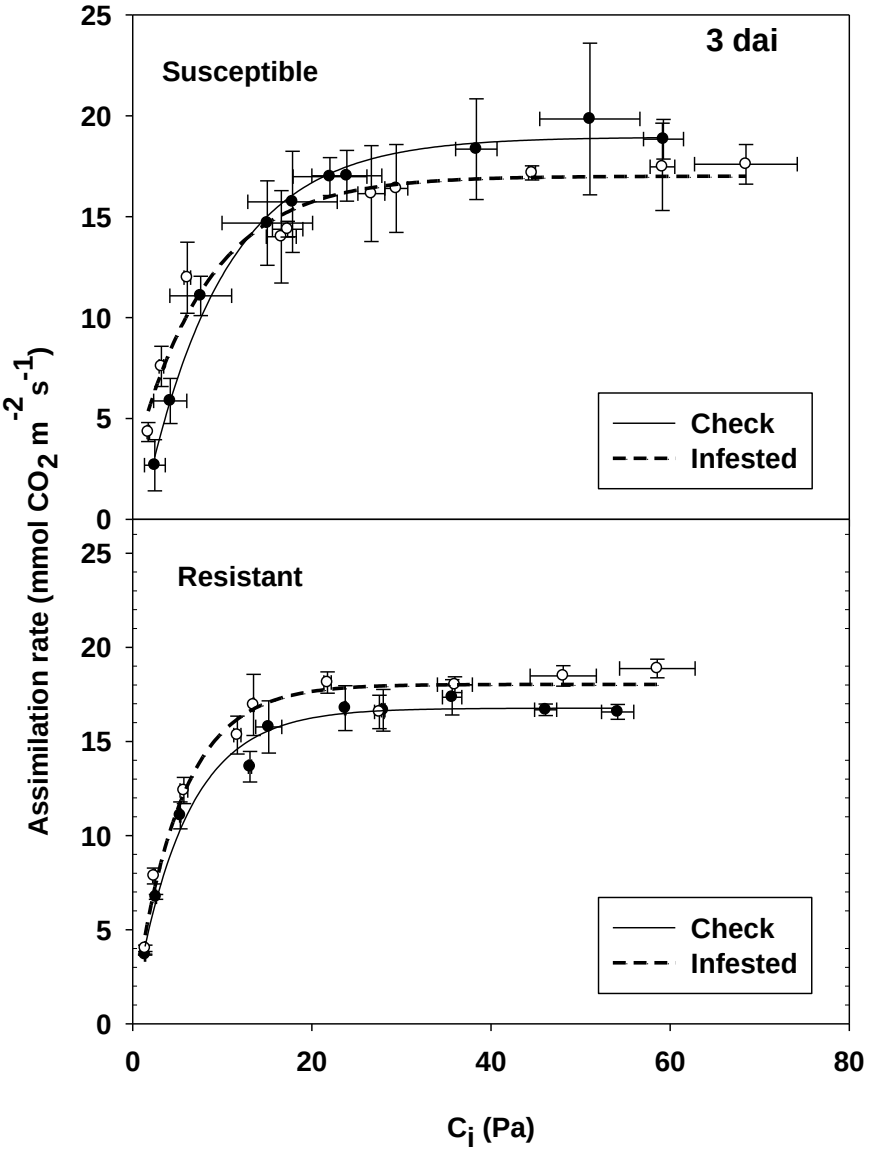

Figure 2. Assimilation (μmol CO_2 m^{-2} s^{-1}) verses intercellular CO_2 concentration (Ci) in Pascals (Pa) for susceptible (KS585) and resistant (DKS 37-07) at 3 d after *M. sacchari* infestation.

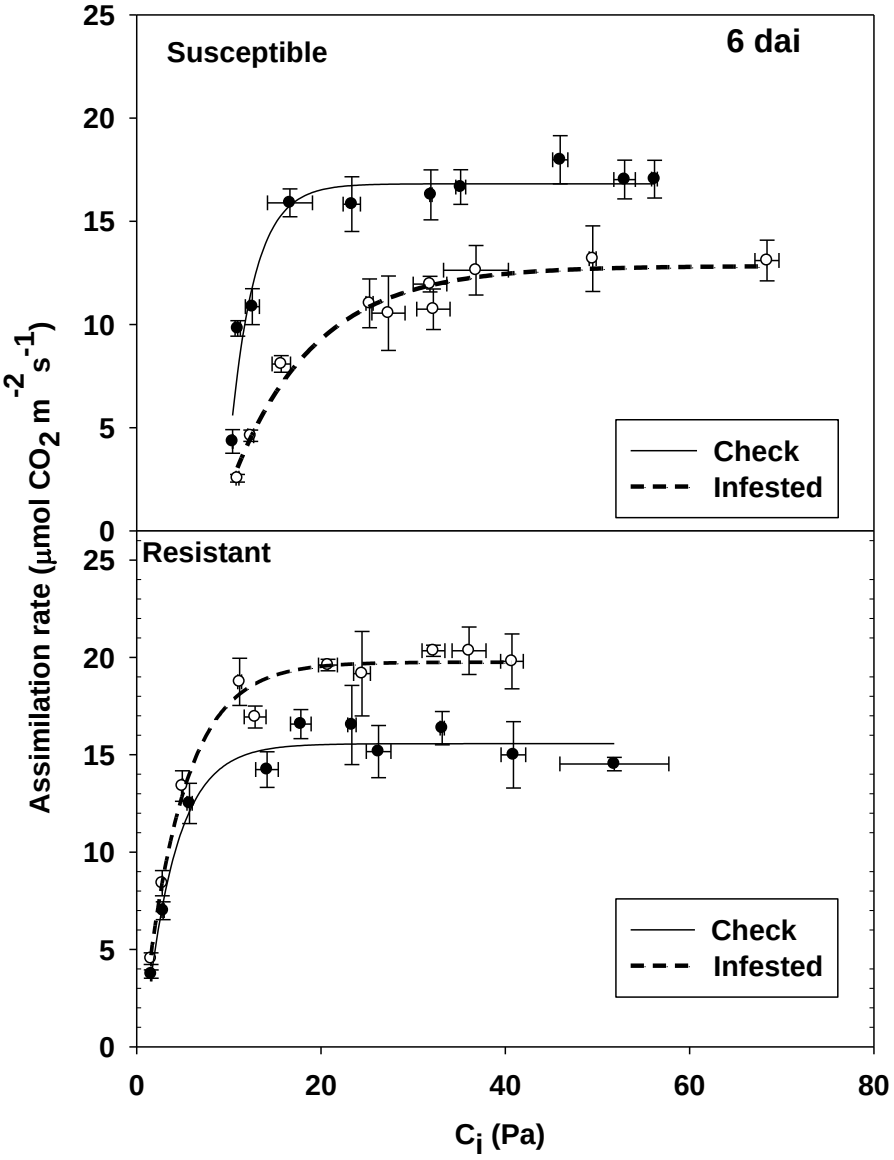

Figure 3. Assimilation (μmol CO_2 m^{-2} s^{-1}) verses intercellular CO_2 concentration (Ci) in Pascals (Pa) for susceptible (KS585) and resistant (DKS 37-07) at 6 d after *M. sacchari* infestation.

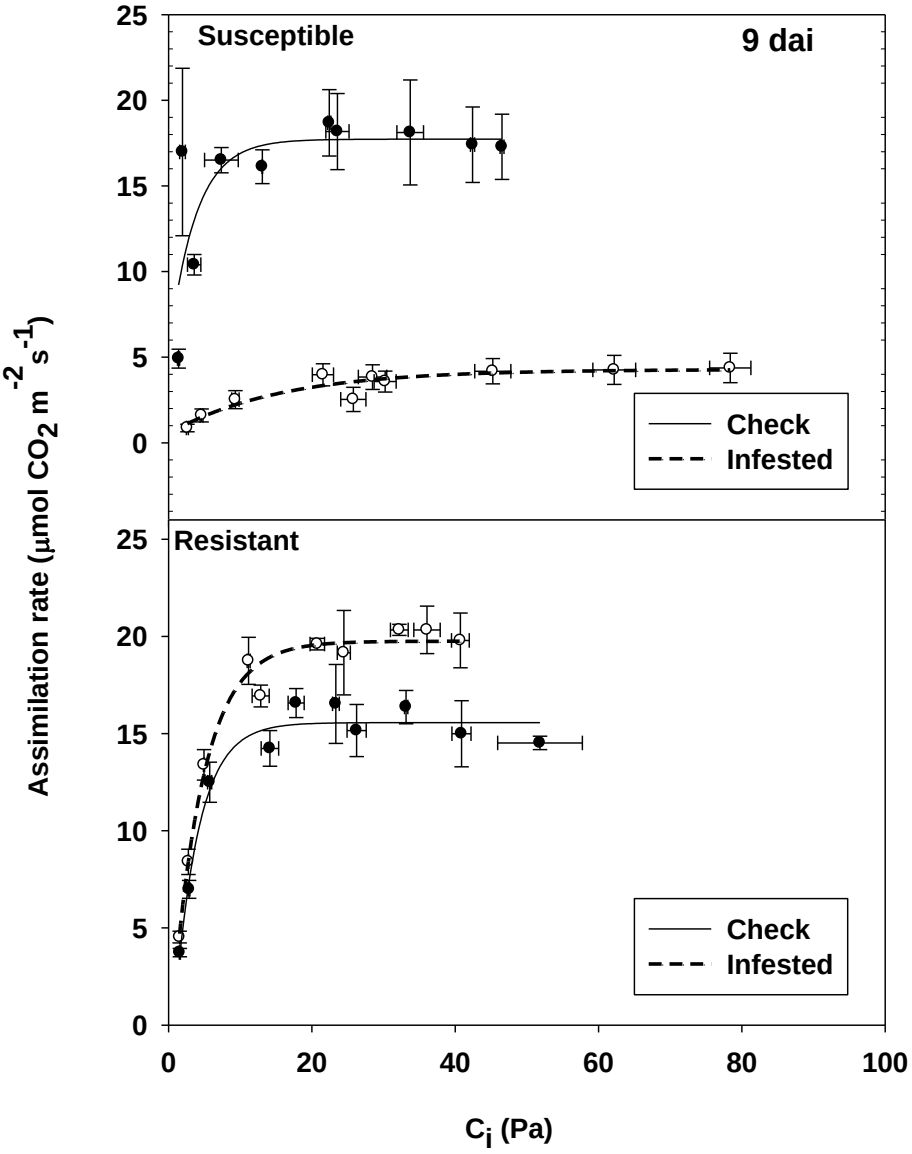

Figure 4. Assimilation (μmol CO_2 m^{-2} s^{-1}) verses intercellular CO_2 concentration (Ci) in Pascals (Pa) for susceptible (KS585) and resistant (DKS 37-07) at 9 d after *M. sacchari* infestation.

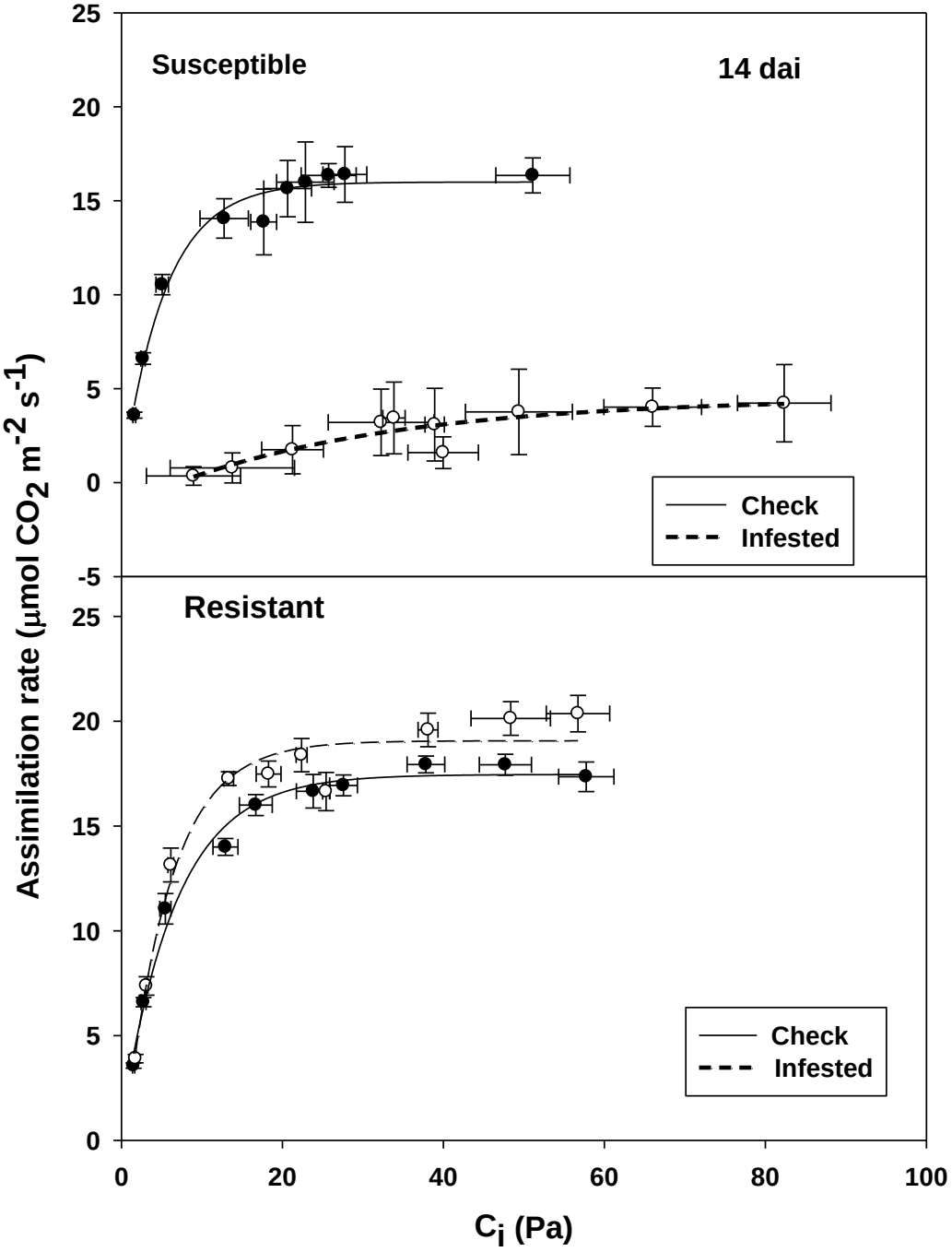

Figure 5. Assimilation (µmol CO_2 m^{-2} s^{-1}) verses intercellular CO_2 concentration (Ci) in Pascals (Pa) for susceptible (KS585) and resistant (DKS 37-07) at 14 d after *M. sacchari* infestation.

CHAPTER IV

Categories of Resistance to Sugarcane Aphid (Hemiptera: Aphididae) among Sorghum Genotypes

Note: Published in Journal of Economic Entomology

Citation:

Paudyal, S., J. S. Armstrong, K. L. Giles, M. E. Payton, G. P. Opit, and A. Limaje. 2019. Categories of resistance to sugarcane aphid (Hemiptera: Aphididae) among sorghum genotype sources. J. Econ. Entomol. toz077, https://doi.org/10.1093/jee/toz077

ABSTRACT The sugarcane aphid *Melanaphis sacchari* (Zehnter) has emerged as a potential threat to sorghum (*Sorghum bicolor* (L.) Moench) production in the United States. Since the late summer of 2013, finding and advancing *M. sacchari* resistant germplasm has been a priority for all stakeholders involved. We evaluated twenty-three sorghum genotypes for resistance to the sugarcane aphid by testing for tolerance, and antixenosis. In addition, nine sorghum germplasm were evaluated for the expression of antibiosis. Free-choice and no-choice tests were conducted to explore the functional categories of resistance. Levels of resistance to *M. sacchari* were compared with the known resistant 'TX 2783' and the susceptible 'KS 585'. Sorghum entries AG1201, AG1301, W844-E, and DKS 37-07 were identified as expressing tolerance, antibiosis, and antixenosis, while H13073 expressed antibiosis and GW1489 expressed both tolerance and antibiosis. These resistant sorghums identified during this study will have a significant impact on reducing economic damage from the sugarcane aphid infestations.

KEY WORDS *Melanaphis sacchari*, sorghum, antibiosis, antixenosis, tolerance

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal crop used as grain, fiber, and fodder (Young and Teetes 1977, FAO 1995). Sorghum production in the United States is currently facing a serious threat from the attack of a new aphid pest, the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) (Armstrong et al. 2015, Elliott et al. 2017). Although *M. sacchari* was first detected on sugarcane (*Saccharun officinarum* L.) in United States (Hall 1987), it was first reported on grain sorghum near Beaumont, TX, in 2013 (Knutson et al. 2016, Bowling et al. 2016). Since its detection on sorghum in Texas, it has been progressively expanding its range and currently reported in 17 states including most of the southern U.S. (Bayoumy et al. 2016, Knutson et al. 2016). *Melanaphis sacchari* has also been reported on johnsongrass (*Sorghum halepense* (L.) Pers.) and Sudangrass (*Sorghum verticilliflorum* (Steud.) Stapf.) (Hall 1987, White et al. 2001, Armstrong et al. 2015).

Melanaphis sacchari is capable of sucking sap from leaves and stems (Villanueva et al. 2014, Bowling et al.2016), but it has a tendency to colonize the underside of lower leaves, and the colonies move upward eventually making their way to the sorghum head (Singh et al. 2004, Bayoumy et al. 2016). Colonies increase rapidly in warm dry weather causing plant injury from significant loss of plant assimilates and heavily infested heads may not produce seed especially when sorghum is in anthesis. If the aphid colonizes the head before anthesis, the flower may not develop into seed, or the seed may be of poor quality. Damage caused by *M. sacchari* infestations is not only due to feeding on leaves and the grain head, but also due to honey dew production which fosters the growth of sooty molds that interfere with chlorophyll production (Singh et al. 2004, Elliott et al.

2015) and making it difficult to mechanically harvest sorghum (Bowling et al. 2016, Brewer et al. 2017).

Deploying resistant sorghum germplasm against *M. sacchari* remains both a short and long-term priority and is considered a cornerstone of future sustainable management programs. There are three functional categories of resistance: antibiosis, antixenosis, and tolerance (Painter 1951, Singh et al. 2004). Antibiosis is characterized by the host plant having an adverse effect on the biology of the aphid including overall reproduction or growth and survival of the aphid. Antixenosis, also known as non-preference is simply defined by a lack of preference for the host because of a morphological and/or chemical factor. Tolerance is considered a plant trait that allows plant growth and the production of grain despite insect feeding (Painter 1951). Research by Girma et al. (1998) advanced the knowledge of the relationship between the chlorophyll loss and the degree of tolerance in sorghum to aphids. Chlorophyll loss has been measured in many recent studies to determine the relationship between aphids and plant tolerance (Flinn et al. 2001, Lage et al. 2003).

Interestingly, some of the same sources of sorghum germplasm that have been demonstrated resistant to the greenbug (*Schizaphis graminum* Randoni) are also resistant to *M. sacchari* (Armstrong et al. 2015, Armstrong et al. 2017, Mbulwe et al. 2016). Greenbug was identified as a pest of sorghum in 1968 (Harvey and Hackerott 1969), several different biotypes that attack sorghum have been described (Burd and Porter 2006). Cross resistant sources of sorghum (i.e. resistant to the greenbug and subsequently also found to be resistant to *M. sacchari*) include SC110, Capbam, and PI 550610 (Armstrong et al. 2015). The sorghum sources SC110 and Capbam originated from South

Africa and Russia, respectively and were found to have resistance to the greenbug (Peterson et al. 1984). Another source of resistance PI 55610 which was resistant to greenbug biotypes C and E was used to develop DKS 37-07 which is used in this study (Peterson et al. 1996, Limaje et al. 2018). However, little is known about whether the entries developed from these sources are resistant to *M. sacchari*. Armstrong et al. (2015) identified eight resistant parental lines that can be used in breeding programs for *M. sacchari* resistance. In addition, two lines (B11055 and R13219) with high degrees of tolerance, antixenosis, and antibiosis were tested, registered and released as Tx3408 and Tx3409 (respectively) from the Texas A&M sorghum breeding program (Mbulwe et al 2016). Further research by Limaje et al. (2018) identified two additional parental lines (R.11143 and R.11259), that express high levels of tolerance and antibiosis to *M. sacchari* and have recently been registered and released as R. LBK1 and R. LBK2 (respectively) by the USDA-ARS sorghum genetics program in Lubbock, TX (Hayes et al. 2018). These resistant sorghum genotypes are promising for future *M. sacchari* management programs, particularly those that can be deployed in locally adapted cultivars.

The purpose of this research was to identify sources of *M. sacchari* resistance in sorghum by using conventional screening methods. Resistant sorghum genotypes identified in this study will hasten the development of commercially suitable sorghum varieties for sorghum growers in the Southern U.S. Additionally, the mechanism(s) of resistance were determined for sorghum genotypes which were categorized as resistant. The effect of each resistant category was combined to obtain plant resistance index (Inayatullah et al. 1990) for each sorghum entry.

Materials and Methods

Aphid Cultures. *Melanaphis sacchari* used in this study were collected from near Bay City, Matagorda County, TX in August of 2013 and have since been maintained on susceptible sorghum RTx7000 at the USDA-ARS Laboratory in Stillwater, OK on seedlings in the greenhouse where T-6 fluorescent lighting was used as an additional light source with a daytime and nighttime temperature of 25-31^{o}C and 19-24 ^{o}C, respectively and 16:8 (L: D) photoperiod. The seedlings were grown in 4.4-L pots each fitted with 45-cm tall ×16-cm diameter cylinder of Lexan TM (SABIC Polymershapes, Tulsa, OK) covered at the top with organdy cloth for ventilation and to prevent SCA from escaping.

Sorghum Genotypes. Sorghum entries and sources used in this study are listed in Table 1. The Tx2783 was used as a known resistant check, and was released by Texas A&M Experiment Station in Lubbock, TX (Peterson et al. 1984), and identified as resistant to *M. sacchari* by Armstrong et al. (2015). The sorghum genotype 'KS 585' was obtained from Chromatin® Seeds and used as a susceptible. All other entries listed were unknown in terms of resistance and susceptibility to *M. sacchari*.

Resistance evaluations.

Tolerance. A free-choice test was conducted in order to determine the relative level of tolerance among the germplasm entries. All lines were compared with known resistant and susceptible cultivars, TX2783, and KS 585, respectively. Aphid damage rating, plant height, number of leaves, and chlorophyll loss were used to assess the level of tolerance in each of the sorghum entries. Damage ratings were assigned when approximately 85% of the susceptible checks (KS 585) were dead. Plants were rated

using a scale of 1 to 9 where: 1 is a healthy plant with no damage, and 9 is a dead plant (Starks and Burton 1977). Experiments were conducted in a greenhouse maintained at temperature of 21–31°C and T-8 fluorescent lights for 24 h (a photoperiod of 14:10 L:D). Twenty-three sorghum entries with twenty replications were planted in a completely randomized block design using plastic seed trays (Growers Supply, Dyersville, IA 52042) with 128 individual cells (4 trays were used for infested treatment). There were eight replications for the uninfested control (2 trays were used for uninfested treatment). One replication consisted of sorghum genotypes that were randomly assigned to the rows and planted at the rate of 2 seeds per cells. Plastic trays had three layers of media: potting soil, fritting clay, and sand (bottom to top respectively). When seedlings reached the 2–3 leaf stages (~7 d), they were thinned to one in each cell and infested with *M. sacchari* with mixed ages by placing infested leaves from the culture in between the rows of each plastic tray in the aphid-infested treatment to ensure high aphid pressure (Sparks and Burton, 1977). Aphid-infested and non-infested trays were kept separate inside the wooden cage (height=196 cm, width=60 cm, length=180 cm) covered with fine mesh to prevent aphid movement. Plant measurements (plant height, number of leaves, chlorophyll content, and damage ratings) were compared among the genotypes. Chlorophyll content was recorded using a SPAD model 502 chlorophyll meter (Minolta, Tokyo, Japan). Three readings from the plants were recorded and averaged to get a SPAD value for all plants. SPAD values from infested and uninfested plants were calculated by the SPAD index = (C – T)/C (Deol et al. 1997, Akbar et al. 2010), where C = SPAD value for non-infested leaf tissue, and T = SPAD unit value for infested leaf tissue. To calculate chlorophyll loss percentage, the SPAD index values were multiplied by 100.

Mixed model analyses (PROC MIXED, SAS Institute 2010) were conducted to detect the differences in damage rating, plant height, chlorophyll loss, and number of leaves; replications were considered as random effects. When appropriate, means were separated using protected pairwise comparisons (DIFF option in a LSMEANS statement).

Antixenosis. In this test, sorghum genotypes listed in Table 1 were planted in 20.3-cm-diameter pots in a circular pattern. Each pot (total of 10) was filled with potting soil, fritting clay, and sand (bottom to top respectively) and planted with 23 lines that were randomized to avoid any environmental effects. Two seeds of each entry were placed around the edge of the circular pot in each depression at a distance of 2–3 cm apart, where seedlings were thinned to one plant when they reached 2–3 leaf stages (i.e. 15–20 cm height). The entries were infested at this stage by placing an estimated 1,000 aphids in the center of each of the pot. Each pot after infestation was individually caged in a wooden cage covered with fine mesh (height=37 cm, width=58 cm, length=60 cm). The number of aphids per plant was recorded at 24, 48, and 72 h post infestation.

Mixed model analyses (PROC MIXED, SAS Institute 2009) were conducted to detect the differences in *M. sacchari* preference among the sorghum genotypes. Comparison of number of aphids in the antixenosis test were conducted following square root transformation to correct for heterogeneous variances and the lack of normality in the aphid numbers. When appropriate, means were separated using protected pairwise comparisons (DIFF option in a LSMEANS statement).

Antibiosis (No-choice experiments). *No-choice experiment 1.* In the no-choice test, 23 sorghum genotypes served as a treatment plants and 23 others of the same genotypes were controls. Each of the genotypes in both treatment and control plants had 5

replications. Experiment was conducted in a greenhouse maintained at temperature of 21–31°C and T-8 fluorescent lights for 24 h. Seeds were placed in the depression made within separate cone-containers™ (model SC10, S7S greenhouse supply, Tangent, Oregon 97389) filled with three layers of media as described above. Individual seedlings (treatment plants) at the 2–3 leaf stage were infested with 20 apterous mature aphids per plant. Control seedlings with five replications were left uninfested. Immediately after infesting, plants were caged using PVC plastic (Lexan TM SABIC Polymershapes, Tulsa, OK) cylinder cages that were 45-cm tall × 8-cm diameter.) The tops of the cylinder cages were covered with organdy cloth. Parameters such as, damage rating, plant height, number of leaves were recorded when ≥85% of the susceptible plants (KS 585) were dead. In Addition, chlorophyll content was recorded using a SPAD similarly as free-choice test. Three readings from the plants were recorded and averaged to get a SPAD value for all plants. SPAD values from infested and uninfested plants were calculated by the SPAD index = (C – T)/C (Deol et al. 1997, Akbar et al. 2010), where C = SPAD value for non-infested leaf tissue, and T = SPAD unit value for infested leaf tissue. To calculate chlorophyll loss percentage, the SPAD index values were multiplied by 100.

No-choice experiment 2. Life table studies were conducted to determine the antibiosis effects of nine sorghum cultivars chosen from the results of the free-choice and no-choice experiments. Media for growing seedlings, cone size, and cages design were adopted from Armstrong et al. (2017). During screening for antibiosis on sorghum, two seeds of each germplasm were planted in a container with three layers of media. When plants were at the three-leaf stage in containers, they were thinned to one plant each and infested with one mature *M. sacchari* female maintained as explained above. After 24 h,

all aphids were removed with a fine camel-hair brush, leaving one first instar nymph on the leaf. Each plant in a cage was observed systematically each day at approximately the same time. Plants were kept in a growth chamber under 14:10 (L: D) photoperiod using T6 white fluorescent bulbs. The temperature in each growth chamber was maintained at 26.6^{o}C (~80 ^{o}F). The experimental design for this study was a completely randomized design with six replications. Life table parameters such as pre-reproductive period, d, (the time taken for the nymph to reach reproductive maturity), Md (the number of progeny produced for a time equivalent to d), total fecundity, and longevity were recorded. The intrinsic rate of increase (r_m) was estimated using the formula developed by Wyatt and White (1977): r_m= 0.738 (logeM_d)/d. The time required for a population to complete one generation (T) was calculated by using the formula Td= d/0.738.

Among entries, pre-reproductive period (d), M_d, intrinsic rate of increase (r_m), time required for a population to complete one generation (T) were analyzed using PROC MIXED. Means of all variables were separated using protected pairwise comparisons (DIFF option in a LSMEANS statement).

Plant Resistance Index (PRI). The plant resistance index combines three resistance categories of antibiosis, antixenosis, and tolerance into one value for total resistance expression used in many studies (Webster et al. 1987, Inayatullah et al. 1990, Webster and Porter 2000, Lage et al. 2003, Razmjou et al. 2012). Our data from each resistance category from 9 sorghum genotypes (selected for antibiosis assay (no-choice experiment 2), Tolerance (X= damage ratings), antixenosis (Y= number of aphids per plant), and antibiosis (Z1= damage rating from no-choice experiment 1 and Z2 = number of nymph per female from no-choice experiment 2), were normalized by dividing them

by the highest mean within each category. Normalized X (tolerance index), Y (antixenosis index), and Z (antibiosis index) values were used to calculate PRI values by using the formula: PRI= 1/XYZ (Razmjou et al. 2012).

Results

Tolerance. In the free-choice tolerance assay, differences among genotypes were highly significant for damage ratings. The damage rating was highest for H13-0086, 9.0 (Fig. 1). There was no significant difference in damage ratings between H13-0086 and the susceptible check (KS 585) indicating they were both highly susceptible. The genotypes AG1201, AG1203, W844-E, and DKS 37-07 exhibited high expression of resistance with damage ratings of ≤3.0, and were significantly lower than the resistant check TX2783 that had a damage rating of 3.5. Moderately resistant genotypes (>3.0 to ≤ 6.0) were identified for OL2042, 12GS9017, 12GS9011, 12GS9012, and 12GS9016. The genotypes 12GS9023, PI 550610, AG1101, and GW1456 were classified as susceptible (> 6.0).

Chlorophyll Loss from *M. sacchari* damage varied greatly among entries ranging from 2.1% (DKS 37-07) to 95.2% (H13-0086) (Fig. 1). The genotypes with <20% chlorophyll loss were AG1201, AG1203, ADV 95157, W844-E, and DKS 37-07 in the free-choice evaluation. Damage ratings and chlorophyll loss were highly correlated (r=0.82 (free-choice assay) P<0.0001), indicating *M. sacchari* accounted for extensive loss of chlorophyll in the susceptible genotypes.

Antixenosis. Among the tested genotypes, no differences were observed for number of *M. sacchari* after 24, 48, and 72 h (F=1.39; df = 2, 441; P=0.25). However,

significant differences for the number of aphids were observed among the 23 genotypes tested (Table 2). Sorghums that appeared to be the most preferred based on the high numbers of *M. sacchari* were GW1456, KS585 (susceptible check), and 12GS9023, where number of aphids counted exceeded 32 per plant. The genotypes, 12GS9012, OL2042, and 12GS9017 had fewer than 6 aphids per plant for all three time periods. Other genotypes with significantly fewer aphids than the KS 585 (susceptible check) were DKS 37-07, TX2783, Ag1301, AG 1201, and H130373.

Antibiosis. *No-choice Experiment 1.* Differences among genotypes in the no-choice assay were significant for number of aphids, damage ratings, and chlorophyll loss (Table 3). The total number of aphids at 14 d post-infestation (dpi) varied from 0 to 255. However, the 0 aphids in some genotypes may reflect interpretation confounded by plant death. Entires 12GS9011 and H13-0086, had no aphids, however, the damage rating was >6. The damage ratings of all genotypes were similar to ratings of the free-choice assay except one genotype, 12GS9041 (Figure 1 and Table 1). Therefore, the reason behind the inconsistency of the damage rating of 12GS9041 in free-choice (5.1) and no-choice assay (3.0) was investigated using three genotypes (12GS9041, KS585, and TX2783) where another no-choice test was conducted. Five out of twenty aphids had established on 12GS9041 at 2 dpi and then increased slowly when compared with the susceptible check, where the number of aphids increased continuously and peaked at 6 dpi and decreased to 0 with damage rating of 9 at 8 dpi (Fig. 3). Due to non-preference of 12GS9041, only 5 aphids had established by 2 dpi, however, the number increased slowly and leaves ultimately sustained a damage rating of 7 at 20 dpi. Clearly, sometimes a conventional

screening assay may provide unreliable measurements of tolerance when compared with susceptible check that dies too quickly.

No-choice Experiment 2. Among the 23 lines that were used in the tolerance and antixenosis assays, seven genotypes were consistent with low damage ratings and low number of aphids when compared to the susceptible check, and were selected to further describe potential antibiosis expression. Among all genotypes tested, there were highly significant differences in *M. sacchari* pre-reproductive periods (*d*), total reproduction, number of nymph per day, and longevity among the nine genotypes (Table 4). The pre-reproductive period was highest on DKS 37-07 (8.2) and lowest on WSH117 (5.4). Reproduction was highest on KS 585 (63.6) followed by WSH117 (62.1) and the total fecundity on the other seven genotypes tested were significantly lower than these two. The mean number of nymphs per day per female produced on KS 585 and WSH117 was more than double that of DKS 37-07, AG1201, GW1489, and H130373, indicating relatively strong antibiotic effects in the latter sorghum genotypes. Adult longevity of *M. sacchari* was affected significantly by sorghum entries (Table 4; F=4.5; df=8,71; P=0.0002). The adult female lived longest in WSH 117 and shortest in H130373.

In addition, the intrinsic rate of increase (r_m), population doubling time (T), time required for a *M. sacchari* to complete one generation (Td) also showed there were significant differences among the genotypes (Table 5). The r_m value for *M. sacchari* calculated for the nine genotypes ranged from 0.15 (AG 1201) to 0.4 (KS 585) (Table 5). Intrinsic rate of increase (r_m) of the seven genotypes that had been selected for the antibiosis test were lower than that of the susceptible variety. Similarly, population doubling time was two-fold shorter for H130373, AG1201, and DKS 37-07 than the

susceptible check (Fig. 2). Moreover, the mean time required for a *M. sacchari* to complete one generation (Td) varied from approximately 7 to 11 d and was longest in DKS 37-07 and AG 1201.

Plant Resistance Index (PRI). The higher PRI values on DKS 37-07 (57.97 and 70.18), AG1201 (74.97 and 74.30) indicated that these lines are highly resistant in comparison with other genotypes having the antibiosis, tolerance, and antixenosis characteristics. Moreover, genotypes such as W844-E, TX2783, AG1203, H130373, and GW1489 have relatively higher PRI compared with susceptible check.

Discussion.

Development of aphid resistant sorghum is an essential step towards more sustainable IPM programs (Smith 2005, Armstrong et al. 2017). In relation to *M. sacchari*, this study has identified resistant entries and resistance categories (tolerance, antixenosis, and antibiosis) for 23 sorghum genotypes. Data showed that aphids find their host within a short period of time and do not leave those plants that they have selected (no significant difference in number of aphids in 24, 48, and 72 h). Some genotypes in this study revealed that there is strong non-preference by aphids and this is likely due to antixenosis properties. However, *M. sacchari* readily fed on sorghum genotypes that appeared to exhibit antixenosis in the no-choice assay.

Determination of tolerance to aphids in cereal plants based on SPAD leaf chlorophyll loss estimate has been described in many studies (Deol et al. 1997, Flinn et al. 2001, Lage et al. 2003, Akbar et al. 2010). In fact, SPAD readings require less labor and time and provide a quantitative measure of injury compared to damage ratings which

can be highly subjective. Maintaining a relatively high chlorophyll content despite infestation is considered a good indicator of plant tolerance (Lage et al. 2003). In this study, genotypes with lower damage ratings (<4) had lower chlorophyll loss (%) and grew taller hence indicated some level of tolerance.

Categories of resistance to *M. sacchari* have previously been documented in grain and forage sorghum by Armstrong et al. (2017). Additionally, Limaje et al. (2018) reported that R.11143 and R.11259 developed by USDA_ARS in Lubbock, TX displayed high level of antibiosis and tolerance. Findings of this study corroborate results by Armstrong et al. (2017) and Limaje et al. (2018) in that the resistant check TX2783 also had high levels of tolerance and antibiosis. However, the highest levels of antibiosis, tolerance and antixenosis were observed for DKS 37-07 and AG1201, which have great potential to become important sources of resistance to *M. sacchari* in sorghum breeding programs. Additionally, the genotypes W844-E, GW1489, TX2783, AG1203, W7051, and H130373 could be interesting choices for resistance management due to their high levels of tolerance; they also have higher antibiosis and antixenosis levels than the susceptible check.

Significant differences in total aphid fecundity among the genotypes found in this study may be explained by host unsuitability. However, the r_m value of insects feeding on plants gives a better representation of antibiotic effects (Webster and Porter 2000, Lage et al. 2003, Smith, 2005). Lower r_m values in the sorghum genotypes DKS 37-07, AG1201, AG1203 and H130373 indicate that delays and/or low reproduction of *M. sacchari* could likely result in reduced plant damage.

The weighted PRI index is a simple way to explain and standardize the overall expected effects of plant resistance where multiple functional categories of resistance can occur (Webster et al. 1987, Inayatullah et al. 1990, Webster and Porter 2000, Lage et al. 2003, Razmjou et al. 2012, Girvin et al. 2017). There are several advantages of having genotypes expressing more than one category of resistance. Tolerant plants are known to sustain lower selection pressure to insect attack, and yield is less affected by infestations. However, tolerance to *M. sacchari* may result in large amount of honeydew and sooty mold which hinder harvesting. For this aphid pest, it could be more advantageous to grow cultivars that have a combination of antibiosis effects that limit population growth and tolerance traits that limit plant damage. Based on this study, genotypes DKS 37-07, AG1201, W844-E, and H130373 have lower antibiosis and tolerance indices with higher overall PRI values. Most of the resistant sorghum genotypes studied here have already been released as varieties offering varying degree of antibiosis, tolerance, and antixenosis that will benefit sorghum producers as one more tool in the arsenal for reducing *M. sacchari* infestations. However, there is need to continue screening sorghum lines that may offer potential resistance to different *M. sacchari* biotypes (Nibouche et al. 2018).

Acknowledgements

We thank B. Driskel and H. Baker for excellent technical support. This research was supported in part by Oklahoma State University under project OKLO2935.

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Table 1. Sorghum genotypes evaluated for resistance against sugarcane aphid

Entry	Genotypes	Obtained from	Maturity	Seed Color
1	12GS9041	Chromatin Inc	Medium	Bronze
2	H130373	Chromatin Inc	Medium-Full	Bronze
3	OL2042	Chromatin Inc	Medium-Full	Bronze
4	12GS9017	Chromatin Inc	Medium-Full	Bronze
5	12GS9023	Chromatin Inc	Medium-Early	White
6	12GS9011	Chromatin Inc	Early	Bronze
7	12GS9012	Chromatin Inc	Medium-Early	Cream
8	H13-0086	Chromatin Inc	N/A	N/A
9	AG 1201	Advanta	Early	Bronze
10	AG 1203	Advanta	Medium-Early	Bronze
11	AG 1301	Advanta	Medium-Early	Cream
12	GW1489	Advanta	68	Red
13	ADV 97157	Advanta	72	Bronze
14	KS 585 (S)	Chromatin Inc.	Medium	Bronze
15	TX 2783	Texas A&M inbred parental line	N/A	N/A
16	PI 550610	USDA (Stillwater, OK)	N/A	N/A
17	AG 1101	Advanta	55	Bronze
18	GW1456	USDA (Stillwater, OK)	N/A	N/A
19	12GS9016	USDA (Stillwater, OK)	N/A	N/A
20	WSH 117 (S)	Warner seed Inc.	N/A	N/A
21	W 7051	Warner seed Inc.	Medium-Full	Red
22	W844-E	Warner seed Inc.	Medium-Full	Red
23	DKS 37-07	USDA (Stillwater, OK)	Medium-Early	N/A

Table 2. Mean (± S.E.) **Number of sugarcane aphids after 24, 48, and 72 h of infesting in antixenosis test**

Number	Sorghum Genotypes	24 h	48 h	72h
1	12GS9041	16.3 ± 4.36 a-g	14.1 ± 3.9 d-h	14.3 ± 4.2 e-i
2	H130373	14 ± 5.52 d-h	12.7 ± 4.5 d-h	10 ± 3.9 f-k
3	OL2042	4.4 ± 0.96 gh	4.2 ± 1.7 h-j	2.9 ± 1.8 i-j
4	12GS9017	5.7 ± 1.52 gh	2.1 ± 0.9 ij	1.7 ± 0.7 k
5	12GS9023	35.4 ± 5.97 ab	43.9 ± 6.7 a	49.2 ± 7.4 a
6	12GS9011	11.8 ± 2.15 a-f	7.9 ± 1.9 e-j	8.1 ± 2.3 f-k
7	12GS9012	3.7 ± 1.22 h	1.5 ± 0.7 j	1.5 ± 0.7 jk
8	H13-0086	23.6 ± 5.47 a-f	28.3 ± 7.0 a-d	38.1 ± 7.2 a-c
9	AG 1201	6.8 ± 2.68 e-f	6.1 ± 2.1 f-j	6.4 ± 2.2 h-k
10	AG 1203	14.4 ± 3.45 c-h	14.2 ± 3.8 c-h	12.2 ± 4.1 e-i
11	AG 1301	8.8 ± 1.59 e-h	11.9 ± 2.1 d-h	9.1 ± 1.9 e-j
12	GW1489	23.2 ± 5.03 a-e	25.4 ± 4.8 a-d	24.7 ± b-e
13	ADV 95157	10.8 ± 3.04 e-h	11.7 ± 3.4 d-i	10.7 ± 3.3 e-k
14	KS 585 (S)	32.3 ± 5.9 a-c	40 ± 8.6 ab	48.3 ± 10.2 ab
15	TX 2783	12.9 ± 2.2 d-h	13.5 ± 3.5 d-h	15 ± 3.9 d-h

Number	Sorghum Genotypes	24 h	48 h	72h
16	PI 550610	21.4 ± 4.2 a-f	26.4 ± 6.0 a-d	24.6 ± 6.5 b-e
17	AG 1101	23.9 ± 7.9 a-f	26.7 ± 9.8 a-f	29 ± 11.2 c-f
18	GW1456	35.6 ± 9.9 a	40.7 ± 11.4 ab	37.5 ± 12.5 a-c
19	12GS9016	15.8 ± 4.7 d-h	22 ± 6.8 b-g	25.1 ± 7.7 c-f
20	WSH117	27.8 ± 5.2 a-d	31.1 ± 4.8 a-c	35.6 ± 6.7 a-c
21	W 7051	24.6 ± 5.5 a-f	25.9 ± 7 a-f	25.1 ± 8.5 c-f
22	W844-E	15 ± 3.19 b-g	15.4 ± 4.6 d-h	14.7 ± 4.8 e-i
23	DKS 37-07	9.2 ± 4.2 f-h	8.2 ± 3.7 g-j	7.3 ± 3.5 g-k

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD

24 h after infestation, df =22, 488; $F = 2.58$; $P = 0.0001$

48 h after infestation, df = 22, 489; $F = 4.39$; $P < 0.0001$

72 h after infestation, df = 22, 489; $F = 5.49$; $P < 0.0001$

Table 3. Damage ratings, plant height, number of aphids, mean leaves per plant and chlorophyll loss for the sorghum lines infested with sugarcane aphid in a no-choice (antibiosis) assay.

Number	Sorghum Genotypes	Damage Rating (1-9 scale)	Plant Height (cm)	Number of aphid	Mean Leaves/Plant	Chlorophyll loss (%)
1	12GS9041	3.0 ± 0.89 a-c	28.1 ± 1.7c-g	102.0 ± 64.3 b-d	3.8 ± 0.2 d-g	19.2 ± 9.7 a-d
2	H130373	2.8 ± 0.37 ab	20.2 ± 2.1 g-j	255.0 ± 74.4 a	4.0 ± 0.0 c-f	25.4 ± 5.3 b-d
3	OL2042	4.0 ± 0.32 ab	34.2 ± 2.1 b-d	43.0 ± 10.0 de	4.8 ± 0.2 ab	41.5 ± 5.5 d-f
4	12GS9017	4.6 ± 0.51 ab	27.1 ± 1.9 d-h	48.6 ± 0 de	4.2 ± 0.2 b-e	39.7 ± 4.4 d-f
5	12GS9023	7.2 ± 0.58 fg	16.1 ± 2.2 jk	0 ± 0 e	3.2 ± 0.2 g-i	54.8 ± 8.4 ef
6	12GS9011	3.6 ± 0.51 b-d	34.5 ± 2.5b-d	48.0 ± 11.2 de	4.2 ±0.2 b-e	18.4 ± 5.9 a-d
7	12GS9012	3.2 ± 0.58 a-c	34.5 ± 2.5 b-d	78.5 ± 11.2 b-e	5.0 ± 0.0 a	21.6 ± 2.3 a-d
8	H13-0086	8.8 ± 0.20 g	10.6 ± 2.4 k	0 ± 0 e	2.6 ± 0.4 i	89.4 ± 9.6 g

Number	Sorghum Genotypes	Damage Rating (1-9 scale)	Plant Height (cm)	Number of aphids	Mean Leaves/Plant	Chlorophyll loss (%)
9	AG 1201	2.2 ± 0.51 ab	29.3 ± 4.5 c-f	110.0 ± 23.4 b-d	3.8 ± 0.2 d-g	-0.7 ± 4.1 a
10	AG 1203	3.0 ± 0.84 a-c	19.7 ± 2.2 h-j	121.8 ± 26.7 b-d	3.4 ± 0.2 f-h	0.68 ± 6.4 ab
11	AG 1301	3.6 ± 0.68 b-d	36.4 ± 2.4 a-c	47.6 ± 13.4 de	4.2 ± 0.2 b-e	-2.8 ± 10.7 a
12	GW1489	3.8 ± 1.35 b-d	35.2 ± 2.8 b-d	58.0 ± 9.7 c-e	4.4 ± 0.2 a-d	3.70 ± 7.6 a-c
13	ADV 95157	3.8 ± 1.36 b-d	28.2 ± 5.5 c-g	38.0 ± 10.0 de	3.4 ± 0.6 f-h	21.9 ± 4.1 a-d
14	KS 585 (S)	8.6 ± 0.40 g	23.2 ± 2.3 f-j	67.0 ± 44.2 b-e	3.2 ± 0.2 g-i	67.8 ± 16.2 fg
15	TX 2783	2.2 ± 0.20 ab	32.6 ± 1.9 c-e	54.0 ± 8.2 de	4.0 ± 0 c-f	-4.53 ± 5.1 a
16	PI 550610	7.2 ± 0.37 fg	30 ± 2.9 c-f	155.6 ± 29.2 b	3.0 ± 0 hi	29.2 ± 11.5 c-e
17	AG 1101	7.2 ± 1.11 fg	27.2 ± 1.2 d-h	65.0 ± 42.8 b-e	3.4 ± 0.2 f-h	65.6 ± 20.4 fg
18	GW1456	6.2 ± 0.92 ef	17.4 ± 2.5 i-k	101.4 ± 19.8 d-e	3.2 ± 0.2 g-i	32.5 ± 10.6 de

Number	Sorghum Genotypes	Damage Rating (1-9 scale)	Plant Height (cm)	Number of aphids	Mean Leaves/Plant	Chlorophyll loss (%)
19	12GS9016	5.0 ± 0.32 de	25.6 ± 1.6 e-i	63.8 ± 12.7 b-e	3.8 ± 0.2 d-g	24.7 ± 6.7 b-d
20	WSH117	5.6 ± 0.40 c-e	23.2 ± 0.6 f-j	49.0 ± 13.2 de	3.0 ± 0 hi	25.9 ± 6.5 b-d
21	W 7051	2.6 ± 0.60 ab	36.2 ± 4.9 a-c	152.0 ± 38.9 bc	3.6 ± 0.2 e-h	3.9 ± 7.8 a-c
22	W844-E	2.2 ± 0.20 ab	44.2 ± 5.9 a	157.0 ± 35.4 b	4.6 ± 0.2 a-c	17.9 ± 12.4 a-d
23	DKS 37-07	1.7 ± 0.24 a	42 ± 3.2 ab	70.4 ± 11.8 b-e	4.0 ± 0 c-f	5.03 ± 3.1 a-c

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD

Damage rating, df=22, 92; F=13.11; $P < 0.0001$

Plant height, df= 22, 88; F=7.91; $P < 0.0001$

Number of aphid, df= 22, 92; F=6.51; P<0.0001

Mean leaves/plant, df= 22, 88; F=7.29; $P < 0.0001$

Chlorophyll loss (%), df= 22, 87.1; F= 5.71; $P < 0.0001$

Table 4. Life table parameters for *M. sacchari* developmental statistics on nine sorghum entries.

Sorghum genotypes	Pre-reproductive period (d)	Fecundity	Number of nymphs/d	Longevity (L)
DKS 37-07	8.22 ± 0.5 a	19 ± 1.74 b-d	1.5 ± 0.4 de	17.3 ± 1.9 c-e
AG 1201	7.8 ± 0.4 ab	11.9 ± 4.4 d	1.4 ± 0.4 de	14.7 ± 2.1 e
GW1489	6.9 ± 0.5 bc	13.4 ± 4.1 cd	1.5 ± 0.3 de	15.3 ± 1.8 de
W844-E	6.9 ± 0.6 bc	34.9 ± 6.5 b	3.2 ± 0.4 bc	21.7 ± 1.7 a-c
KS 585 (S)	6.7 ± 0.4 bc	63.6 ± 8 a	5.3 ± 0.6 a	25 ± 1.6 a
TX 2783	6.6 ± 0.3 c	28.1 ± 4.6 b-d	2.1 ± 0.1 de	20.2 ± 1.7 a-d
AG 1203	6.4 ± 0.3 cd	31 ± 6.6 bc	2.5 ± 0.3 bc	17.8 ± 2.4 b-e
H130373	6.4 ± 0.5 cd	13.9 ± 7.3 cd	1.2 ± 0.4 e	13.8 ± 2.0 e
WSH 117	5.4 ± 0.31 d	62.1 ± 10.5 a	4.1 ± 0.5 b	22.9 ± 1.9 ab

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD.

Pre-reproductive period, df=8, 79; F=3.63; P<0.0012

Fecundity (Total nymph), df= 8, 72; F=12.9; P<0.0001

Number of nymphs/d, df= 8, 80; F=12.99; P<0.0001

Longevity (L), df= 8, 71.2; F= 4.55; P=0.0001

Table 5. Life table parameters (Mean ± SE) **for *M. sacchari* developmental statistics on nine sorghum entries.**

Sorghum genotypes	Md	r_m	Td
DKS 37-07	14.6 ± 3.4 bc	0.20 ± 0.04 de	11.1 ± 0.7 a
AG 1201	9.2 ± 2.9 bc	0.15 ± 0.04 e	10.6 ± 0.6 ab
GW1489	8.9 ± 1.9 c	0.23 ± 0.02 c-e	9.4 ± 0.6 bc
W844-E	18.3 ± 2.7 b	0.30 ± 0.04 a-c	9.3 ± 0.7 bc
KS 585 (S)	42.6 ± 6.5 a	0.40 ± 0.02 a	9.1 ± 0.5 bc
TX 2783	10.2 ± 1.1 bc	0.25 ± 0.01 b-d	8.9 ± 0.5 c
AG 1203	17.5 ± 2.5 bc	0.30 ± 0.02 a-c	8.7 ± 0.4 cd
H130373	10.6 ± 4.4 bc	0.17 ± 0.04 de	8.7 ± 0.7 cd
WSH 117	16.0 ± 3.3 bc	0.34 ± 0.04 ab	7.3 ± 0.4 d

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD.

Number of progeny produced for a time equivalent to d (Md), df= 8, 70.3; F= 10.2; P<0.0001

Intrinsic rate of increase (r_m), df= 8, 80; F= 6.15; P<0.0001

Time required for a population to complete one generation (Td), df= 8, 79; F=3.62; P=0.0012

Table 6. Normalized indices for components of resistance and PRI of nine sorghum genotypes against *M. sacchari*.

Sorghum genotypes	Tolerance (X)	Antixenosis (Y)	Antibiosis (No-choice experiment 1(Z1)	Antibiosis (No-choice experiment 2(Z2)	PRI (1)	PRI (2)
DKS 37-07	0.24	0.28	0.20	0.28	74.40	53.14
AG 1201	0.27	0.21	0.25	0.26	70.55	67.83
GW1489	0.35	0.72	0.44	0.30	9.02	13.23
W844-E	0.25	0.46	0.26	0.60	33.44	14.49
KS 585 (S)	1.00	1.00	1.00	1.00	1.00	1.00
TX 2783	0.42	0.40	0.26	0.39	22.89	15.26
AG 1203	0.28	0.45	0.35	0.47	22.68	16.89
H130373	0.41	0.43	0.33	0.23	17.19	24.66
WSH 117	0.72	0.86	0.65	0.77	2.48	2.10

PRI(1) =(1/XYZ1) and PRI(2) = (1/XYZ2), using X= damage rating of sorghum genotypes in free-choice assay from Figure 1; Y=mean number of *M. sacchari* per plant at 24 h after infestation from Table 2; Z1= damage rating of sorghum genotypes in no-choice assay (Table 3); Z2= mean number of nymphs per day (Table 4). Index (X, Y, Z1, and Z2) values determined by dividing mean response by the highest mean rating in each category.

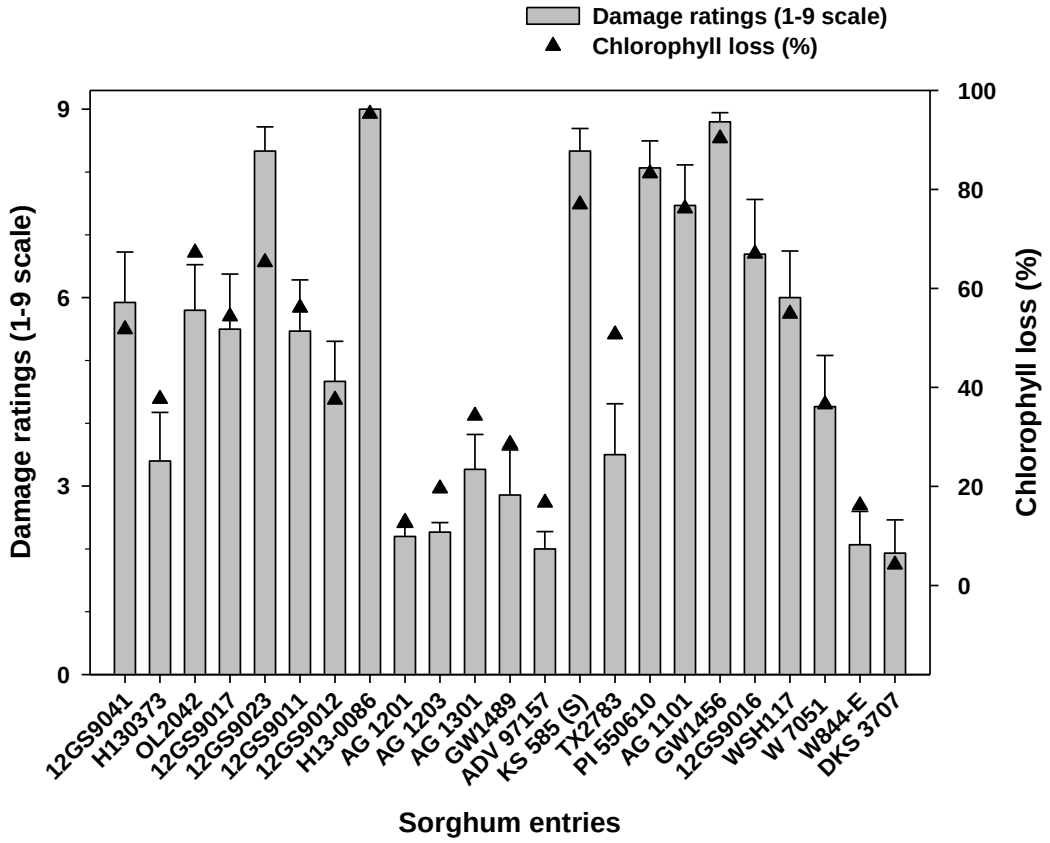

Figure 1. Damage ratings (1-9 scale) and chlorophyll loss percentage in the different sorghum genotypes infested with *M. sacchari* in a free-choice test to determine tolerance. Differences among genotypes were highly significant for the damage rating (df= 22, 297; F= 15.89; P<0.0001) and Chlorophyll loss (%) (df= 22, 296; *F*= 11.34; *P*<0.0001).

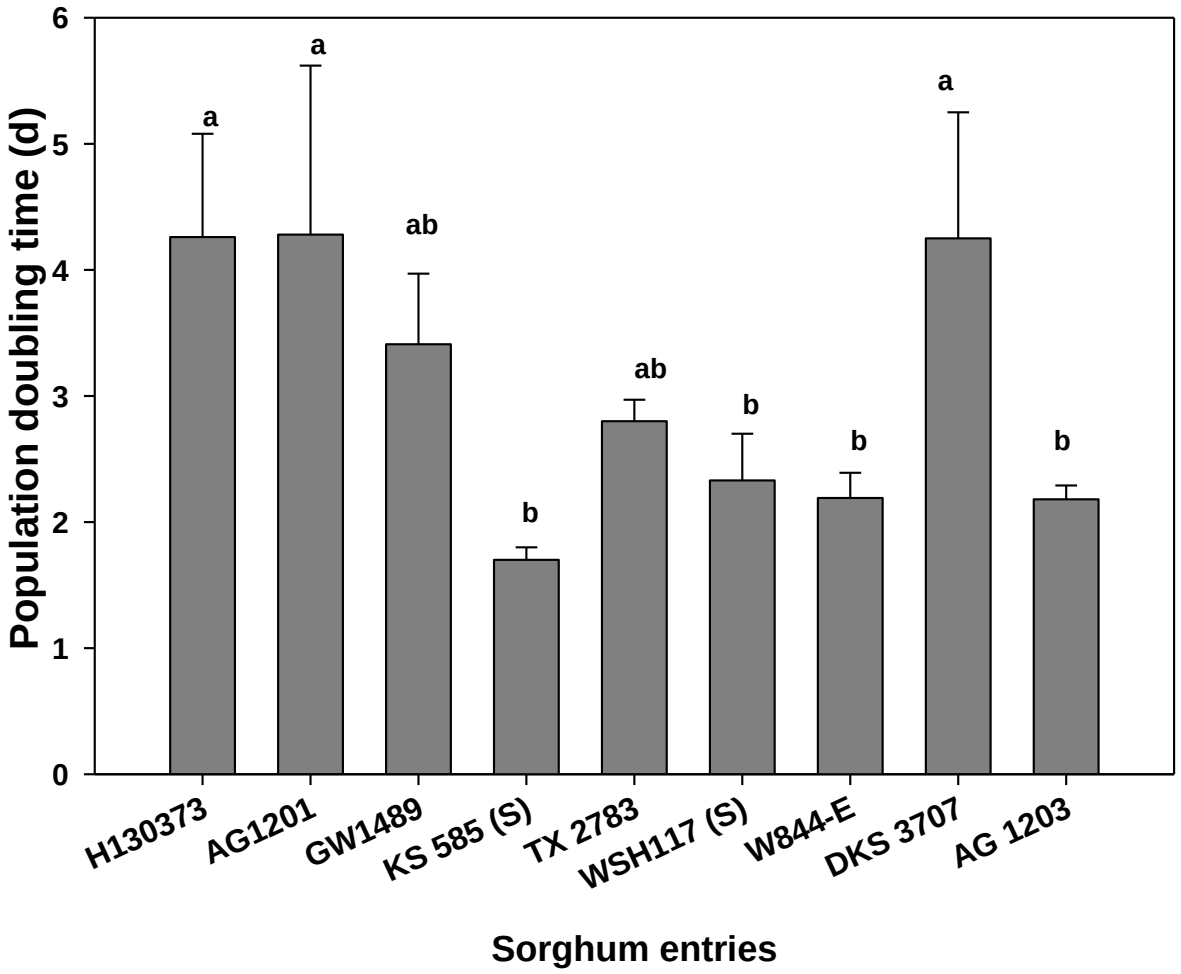

Figure 2. Population doubling time (Dt) in days in the different sorghum genotypes infested with *M. sacchari* in a no-choice test to determine antibiosis. Means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD. Differences among genotypes were highly significant for the population doubling time (df = 8, 73; F=2.85; P=0.0083).

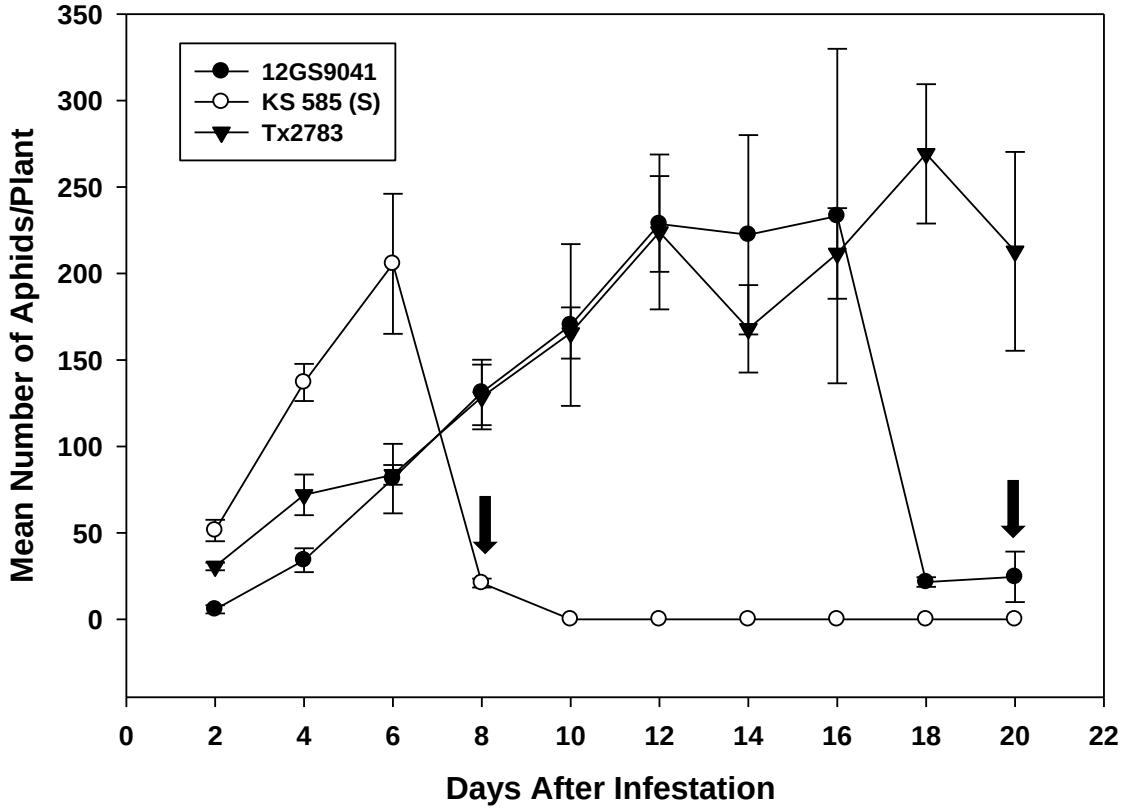

Figure 3. Mean ± SE sugarcane aphid numbers per plant from 2 to 20 d after initial infestation of twenty adult aphids in three genotypes; Down arrow (⇩) indicates that the plants were almost dead with damage rating (>7).

CHAPTER V

Evidence of Host Plant Specialization Among the U.S. Sugarcane Aphid (Hemiptera: Aphididae) Genotypes

ABSTRACT The sugarcane aphid (*Melanaphis sacchari* (Zehnter) (Hemiptera: Aphididae) has become a serious pest in the United States and the number one rated pest of sorghum (*Sorghum bicolor* (L.) Moench) since it was detected in Texas in 2013. Sugarcane aphid was considered only a pest on sugarcane in Florida and Louisiana for over three decades before the 2013 outbreak. Recent studies suggest that the 2013 outbreak in sorghum was because of the introduction of new genotype. The objective of this was to quantify the phenotypic behaviors (host suitability as measured through life table statistics) and correlate with the genetic diversity among sugarcane aphid clones collected from different hosts. We collected a diverse group of sugarcane aphid clones that colonized sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA) and determined host suitability when introduced to five different hosts plants that included a resistant and susceptible grain sorghum, sugarcane, Columbus grass, and Johnsongrass. Sugarcane aphid clones from different hosts and geographical regions varied in performance among plant hosts. The survivorship and reproduction of the sugarcane collected aphid clone (SuSCA) was significantly higher when offered sugarcane (>85%) as compared to other hosts and in contrast, there was negligible survival and reproduction when SoSCA and CoSCA were offered sugarcane as host. Genotyping (conducted by Dr. Karen Harris, USDA-ARS, Tifton, GA) of the aphid clones collected from various hosts with the microsatellite markers indicated that SuSCA was a different MLG (multilocus genotype, MLL-D) when compared to SoSCA and CoSCA, (MLL-F). This study suggests that there exist two different biotypes of the sugarcane aphid within the United States. which are host specific, and that they cannot be distinguished by taxonomic or morphometric characteristics.

KEYWORDS *Melanaphis sacchari*, host-plant specialization, sugarcane aphid genotypes

Introduction

Phytophagous insect species that feed on different species of plants can lead to populations that become more specialized to different hosts over time (Ferrari et al. 2008, Jean and Jean-Christophe 2010, Nosil 2012). Insects on different hosts may experience a diversity of environments, different sets of natural enemies, and different geographic locations which favors divergent selection (Nosil 2004, Ferrari et al. 2008). Additionally, host plants species have different nutritional compositions and secondary metabolites which can further place selection pressure on insects (Guerrieri and Digilio 2008). The term 'ecological speciation' has been used to describe this type of adaptation of a species to various ecological environments and plants as a result of ecologically-based divergent selection (Ferrari et al. 2008, Carletto et al. 2009, Nosil 2012). Consequently, the evolutionary process of adaptation to different ecological environments can produce phenotypic and genetic differences among populations (Nosil 2012).

Phytophagous insects, especially aphids, are known as ecological specialists (Via 1991, Mokhtar et al. 1993, Ferrari et al. 2006, 2008). Pea aphid (*Acyrthrosiphon pisum* Harris) populations feeding on alfalfa and red clover, respectively, are known to be specialized on each of these hosts and show preference to the plant from which they have been collected (Ferrari et al. 2006). These aphids have higher reproduction and survival rates on the host from which they were collected (Via 1999, Ferrari et al. 2008). Similarly, cotton-melon aphid (*Aphis gossypii* Glover) populations have a variable range in ability to reproduce and have host preferences among suitable host plants (Mokhtar et

al. 1993, Agarwala and Chaoudhury 2013, Wang et al. 2016). Several biotypes of greenbugs (*Schizaphis graminum* (Rondani)) and Russian wheat aphids (*Diuraphis noxia* (Mordvilko)) are distinguished on the basis of their reproductive behavior and the ability to damage various wheat genotypes (Wilhoit et al. 1991, Burd et al. 2006, Puterka et al. 2014).

The sugarcane aphid (*Melanaphis sacchari* (Zehntner)) is a relatively new pest of sorghum in the United States with distinct black cornicle tips and black tarsi (Villanueva et al. 2014, Bowling et al. 2016). It has been reported to feed on sugarcane in the U.S (Mead 1978, White et al. 2001), but since its discovery on sorghum in Texas in 2013, it has been rapidly expanding its geographic range (Rodriguez-del- Bosque and Teran 2015, Bowling et al. 2016). *Melanaphis sacchari* have 14 known suitable host plants worldwide which include *Cynodon dactylon* (L.), *Miscanthus sinensis* (L.), *Oryza sativa* (L.), *Panicum colonum*, *Panicum maximum*, *Paspalum sanguinale*, *Pennisetum* sp., *Saccharum officinarum*, *Setaria italic* (L.), *S. bicolor*, *S. halepense* (L.), *S. verticilliflorum* (Steud.), and *Zea mays* (L.) (Singh et al. 2004). To date, the predominant biotype in the United States has a host range limited to *S. bicolor*, *S. halepense*, *Saccharum officinarum*, Sudan grass (*Sorghum drummondii*), and Columbus grass (*Sorghum almum*) (Armstrong et al. 2015, Medina et al. 2016).

Genetic diversity has been examined worldwide and in the Americas for the sugarcane aphid. Nibouche et al. (2015) collected sugarcane aphids from different geographic locations between 2007-2013 and documented five multilocus lineages (MLL) including, MLL-A from Africa, MLL-B from Australia, MLL-C from South America, the Caribbean, Reunion Island, and East Africa, MLL-D from the United

States, and MLL-E from China. Nibouche et al. (2015) also found host specialized lineages of sugarcane aphids collected from sugarcane and wild sorghum (*Sorghum bicolor* subsp. verticilliflorum) in Reunion Island, France. Harris-Shultz et al. (2017) collected populations of *M. sacchari* from sorghum in 2015 from 17 different locations of the United States and concluded that these aphid populations are primarily one asexual clone. This asexual clone was attributed to a new multilocus lineage (MLL-F) and this lineage is considered to threaten the sorghum industry in the United States since 2013 (Nibouche et al. 2018). MLL-F is considered an invader to the Americas from Africa or Asia (Nibouche et al. 2018), and is genetically different than populations collected on sugarcane and Johnsongrass in 2007 from Louisiana and Hawaii (Nibouche et al. 2018). For sugarcane aphid samples collected after 2013 in the continental U.S., sugarcane aphids that were MLL-D were found only on sugarcane, but sugarcane aphid samples that were MLL-F were found on sugarcane, sorghum, and Johnsongrass (Nibouche et al. 2018). Altogether, data from these studies suggested occurrence of host-associated genotypes of the SCA in the United States.

In this study, host plant suitability of sugarcane aphids collected from three primary hosts, sugarcane (*Saccharum* spp.), sorghum (*Sorghum bicolor* (L.) Moench) and Columbus grass (*Sorghum* x *almum*) were compared. Clonal sugarcane aphid collections from these hosts were allowed to feed on sugarcane, susceptible and resistant sorghums, Johnsongrass, and Columbus grass and life table statistics were calculated and compared. Additionally, the results from this study correlated with the genetic study conducted by Dr. Karen Harris, USDA-ARS, Tifton, GA. The genetic diversity of the SCA populations collected from respective host plants were compared using microsatellite markers and

linked to past studies using mitochondrial cytochrome c oxidase subunit I gene (COI) sequencing.

Materials and Methods

Aphid Cultures. The sugarcane aphid clonal lineages were collected from three hosts including sorghum, sugarcane, and Columbus grass. Sugarcane aphids feeding on sorghum were collected from near Bay City, Matagorda County, TX in August of 2013 and have since been maintained on susceptible sorghum RTx7000 in a greenhouse. The greenhouse is equipped with T6 fluorescent lighting (14:10 h (L: D) photoperiod) and temperatures were maintained between 21-31° C. Sugarcane aphids feeding on sugarcane and Columbus grass were collected from Belle Glade Florida (Palm Beach County) and were maintained under previously described greenhouse conditions on the susceptible sugarcane variety CP96-1252 and Columbus grass, respectively. All sugarcane aphid populations were maintained on their respective host plants in 4.4-L pots each fitted with a 45-cm tall ×16-cm diameter cylinder of Lexan TM (SABIC Polymershapes, Tulsa, OK) covered at the top with organdy cloth for ventilation and to prevent aphids from escaping.

Host Transfer Experiments. Sugarcane aphids (SCA) were reared on their primary hosts including, sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA) and transferred to sorghum (susceptible (KS585) and resistant (AG1201)), sugarcane, Johnsongrass, and Columbus grass where life-table parameters and demographic statistics were compared. The susceptible sorghum germplasm (KS585) was obtained from Chromatin Inc. and the resistant sorghum germplasm AG1201 (Paudyal et al. unpublished data) was obtained from Advanta Seeds Pty Ltd. Sugarcane stalk cuttings of cultivar CP96-1252 were obtained from University of Florida at Belle Glade.

Life Table Demography. Sugarcane aphids and host plants were maintained in growth chambers (25 ± 2 ^{0}C, 65 ± 5% RH, and 16:8 h (L: D) photoperiod). Two seeds of each sorghum genotype (susceptible and resistant), Columbus grass and Johnsongrass were planted in cone-tainers™ (model SC10, S7S Greenhouse Supply, Tangent, Oregon) with three layers of media that included potting soil, fritting clay, and sand (bottom to top respectively). When plants reached the three-leaf stage, they were thinned to one plant per cone-tainer™ and infested with one mature apterous *M. sacchari* female from sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA) (8 replications for each host plant and each aphid). For sugarcane, single-bud cuttings were planted into the growth media in individual 4.4L pots. Young shoots with three fully developed leaves were infested with one mature apterous aphid from each host plant: SoSCA, SuSCA, and CoSCA (8 replicates).

Following a 24 h settling period, the adult female and all nymphs were removed, with exception of one nymph. The experiment began with the one-day old nymphs and these were observed daily for their survival and reproduction. All newborn nymphs were removed from each host plant after counting every 24 h, and this process was continued until the founding female aphid died. Life table parameters include pre-reproductive period (d) (the time taken for the nymph to reach reproductive maturity), Md, the number of progeny produced for a time equivalent to d, reproductive period, total fecundity, average daily reproduction and longevity were recorded. The intrinsic rate of increase (r_m) was estimated using the formula developed by Wyatt and White (1977): $r_m = 0.738 (\log_e M_d)/d$.

The three sugarcane aphid populations (SoSCA, SuSCA, and CoSCA), were observed for pre-reproductive period (d), reproductive period, longevity (d), number of nymphs per day, total fecundity, and intrinsic rate of increase (r_m). All variables were compared using PROC MIXED (SAS Institute 2009). Means of all variables were separated using protected pairwise comparisons (DIFF option in a LSMEANS statement) using the Satterthwaite method for the degrees of freedom.

Host plant differentiation. The population dynamics of sugarcane aphids from sorghum (SoSCA) and sugarcane (SuSCA) when reared on five different host plants (susceptible and resistant sorghum, sugarcane, Johnsongrass, and Columbus grass) were investigated in the greenhouse. The greenhouse was equipped with T6 fluorescent lighting (14:10 (L: D) h photoperiod) and the temperature was maintained at 21-31° C. Each host plant had 8 replications for each sugarcane aphid population (SoSCA, SuSCA, and CoSCA). Two seeds of each sorghum genotype (susceptible and resistant), Columbus grass and Johnsongrass were planted in a cone-tainers™ with three layers of media, potting soil, fritting clay, and sand (bottom to top respectively) similar to the previous experiment. When sorghum plants were at the third-leaf stage, plants were thinned to one plant per cone-tainer™ and infested with 20 nymphal aphids. Sugarcane and Columbus grass were infested with 20 aphids from each host when the young shoots had two-three fully emerged leaves. The total number of aphids on each host entry was counted 48 h after infesting and every 48 h thereafter for 12 consecutive days.

Aphid counts were analyzed with mixed model analyses (PROC MIXED, SAS Institute 2009) following a square root transformation to correct for heterogeneous variances and the lack of normality of count response variables. When appropriate, means

were separated using protected pairwise comparisons (DIFF option in a LSMEANS statement, and Satterthwaite option for degrees of freedom).

Sugarcane aphid taxonomy and genotyping. For taxonomic identification, sugarcane aphid clonal colonies that were collected directly from grain sorghum from Texas (SoSCA), sugarcane (SuSCA) and Colombus grass (CoSCA) from Florida, were sent to Dr. Susan Halbert, Florida Department of Agriculture, and Drs. Gary Miller and Christopher Owen with the USDA-ARS Systematic Entomology Laboratory, Beltsville, MD. For genotyping clones of the sugarcane aphid, SoSCA, CoSCA, and SuSCA aphids were collected in 2 mL microcentrifuge tubes and sent to Dr. Karen Harris lab.

Results

Life table demography. Clonal colonies of the sugarcane aphid that were maintained on their original host i.e. sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA) varied widely in their survival, growth, and reproduction when offered a variety of host plants (Table 1, 2, and 3; Fig. 1). Sugarcane aphids collected from sorghum and Columbus grass (SoSCA and CoSCA, respectively) could not reproduce on sugarcane although these populations produced a high number of nymphs per female on sorghum (susceptible), Columbus grass, and Johnsongrass (Figure 1A and 1B). The SoSCA survived for only 9 d on sugarcane, while CoSCA survived for 20 d on sugarcane and both populations had negligible intrinsic rates of increase (Tables 1 and 3) when compared with the remaining host plants. Total fecundity of the SuSCA was highest on sugarcane and significantly lower on the other hosts (Fig 1B). The total number of nymphs per day, pre-reproductive period, reproductive period, intrinsic rate of

increase, and longevity of SuSCA on sorghum were significantly lower than on sugarcane (Table 2). The total number of nymphs per day and pre-reproductive period of SuSCA on Columbus and Johnsongrass were similar to number on sugarcane, however lifespan was shorter with < 21 d for both when compared with 34 d for sugarcane (34 d) (Table 1).

The intrinsic rate of increase (r_m) value for SoSCA and CoSCA on sugarcane was significantly lower (≤ 0.03) than the r_m value for the other hosts (≥0.19; Tables 1 and 3). For SuSCA, the r_m value was highest on sugarcane (0.86) and lowest on resistant sorghum (0.00) (Table 2).

Host plant differentiation. The average reproductive capacity of SoSCA and SuSCA over 12 d on different host plants varied significantly (Figures 2 and 3). Within 2 d after infestation, the SoSCA decreased on sugarcane to the point where there were no survivors on the plants (Fig 2). The number of SoSCA increased with time on resistant sorghum and Columbus grass. However, the number of SoSCA on susceptible sorghum and Johnsongrass was close to zero when these host plants died (Fig 2). The population size of SuSCA on sugarcane increased with time (Fig. 3), and although SuSCA survived on Johnsongrass and Columbus grass, it did not survive on sorghum (Fig 3).

Sugarcane aphid taxonomy and genotyping. Drs. Susan Halbert (Florida Department of Agriculture), Gary Miller, and Christopher Owen (USDA-ARS Systematic Entomology laboratory, Beltsville, MD) reported SoSCA, CoSCA, and SuSCA aphid clonal lineages all were *Melanaphis sacchari* (Zehnter). These clonal lineages had similar antenna process terminalis and the hind tarsas (Blackman and Eastop

1990). Furthermore, they had similar measurement of each antennal segment, ultimate rostral segment, cauda, siphunculus, and the body length.

Genotyping study conducted by Dr. Karen Harris (USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA) found SuSCA sample exhibited unique genotype when compared to SoSCA and CoSCA. She further suggested SuSCA belongs to MLL-F and SoSCA and CoSCA both belongs to MLL-D lineage.

Discussion

Through phenotyping and host switching (Ferrari et al. 2006, Hu et al. 2016) (i.e. sugarcane aphids collected and maintained on sugarcane, Columbus grass, and sorghum, then offered other hosts including Johnsongrass) we were able to show that the sugarcane aphid collected from sugarcane (SuSCA) had significantly reduced survival and reproduction on sorghum, Johnsongrass, and Columbus grass. The sugarcane aphid has been on sugarcane in the United States for over three decades (Hall 1987, White et al. 2001). Sugarcane and sorghum are usually not grown in the same geographical locations, and therefore sorghum might not be part of the host range of the SCA clone occurring on sugarcane. The differing sugarcane aphid type in this study came directly from sugarcane in Belle Glade, Palm Beach County, Florida.

However, offering new host plants revealed that the other two aphid clones (SoSCA and CoSCA) were relatively similar in survival and reproduction, and are likely similar in terms of host plant selection. Sorghum and Johnsongrass are close plant species and are found everywhere throughout the United States, especially in sorghum producing regions. On the other hand, Columbus grass is very prolific and prevalent in the southern

United States, including in transition areas between sugarcane fields and other crops. In Florida, at least two aphid clones (SuSCA and CoSCA) are currently present and when sorghum is planted in experimental fields, it becomes rapidly infested by *M. sacchari*, most likely by individuals coming from Columbus grass.

Based on genotyping analysis, the clone collected from sugarcane are clearly genetically different than the clones collected from sorghum and Columbus grass. The MLL-F lineage of the sugarcane aphid from sorghum reported by Nibouche et al. (2018) is the same sugarcane aphid type (MLG-1) that we collected in this study from sorghum and Columbus grass. Furthermore, the sugarcane aphid clone collected from Florida from sugarcane (MLG-3) corresponds to lineage MLL-D reported by Nibouche et al. (2018).

Based on the results of this research, it is apparent that there are two lineages (MLL-D and MLL-F) of sugarcane aphids in the U.S, which can be differentiated based on their host plant association. This suggests that the sugarcane aphid outbreak that started on sorghum in south Texas in 2013 was caused by the MLL-F type and not by the sugarcane aphid (MLL-D type) that was present on sugarcane in Florida for over three decades (Hall 1987, White et al. 2001). Our results suggest that there exist two different host-specific biotypes of the sugarcane aphid within the United States. Moreover, the origin of the aphid MLL-F type that caused the epidemic outbreak in 2013 on sorghum in the United States remains to be determined.

Acknowledgments

I would like to thank B. Driskel and H. Baker for excellent technical support. I greatly appreciate and thank Dr. Karen Harris, USDA-ARS Crop Genetics and Breeding Research lab for training me in genotyping of aphids. I would also like to thank Dr. Susan Halbert, Florida Department of Agriculture, and Drs. Gary Miller and Christopher Owen with the USDA-ARS Systematic Entomology Laboratory, Beltsville, MD, for helping in the aphid identification.

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Table 1. Life table parameters of sugarcane aphids transferred from sorghum (SoSCA) to five host plants

Host transfer type	Number of nymphs per day	Pre-reproductive period (d)	Reproductive period (Rp)	Intrinsic rate of increase (r_m)	Longevity (L)
Sorghum – Sus. sorghum	5.01 ± 0.28 a	5.5 ± 0.4 a	15.9 ± 0.8 ab	0.48 ± 0.02 d	28.1 ± 1.2 a
Sorghum – Res. Sorghum	1.71 ± 0.33 b	7.3 ± 1.1 a	9.8 ± 2.5 b	0.19 ± 0.03 b	20.1 ± 3.2 b
Sorghum – Sugarcane	0.06 ± 0.04 c	n/a	2.6 ± 1.8 c	0.00 ± 0.00 a	9.0 ± 2.2 c
Sorghum – Johnsongrass	4.92 ± 0.36 a	5.5 ± 0.6 a	18.5 ± 2.2 a	0.45 ± 0.01 d	30.3 ± 2.2 a
Sorghum – Columbus grass	2.15 ± 0.21 b	5.5 ± 0.3 a	22.0 ± 1.1 a	0.33 ± 0.02 c	32.3 ± 1.2 a
	df= 4, 28; *F*=70.16; *P*<0.0001	Df=4, 28; *F*=8.65; *P*=0.072	df=4,28; *F*=21.10; *P*<0.0001	df=4,28; *F*=90.56; *P*<0.0001	df=4, 28; *F*=21.91; *P*<0.0001

Note: Data are Means ± SE. Statistical significance are based on One Way ANOVA. The value with n/a denotes: not available because aphid didn't reproduce (therefore no pre-reproductive period). Values in the same column followed by the same letters are not significantly different at $P < 0.05$ according to DIFF statement in the LSMEANS.

Table 2. Life table parameters of aphids transferred from sugarcane (SuSCA) to five host plants

Host transfer type	Number of nymphs per day	Pre-reproductive period (d)	Reproductive period (Rp)	Intrinsic rate of increase (r_m)	Longevity (L)
Sugarcane – Sus. Sorghum	0.4 ± 0.1 a	4.3 ± 1.6 a	2.9 ± 1.4 a	0.07 ± 0.03 b	11.1 ± 1.6 a
Sugarcane – Res. Sorghum	0.0 ± 0.0 a	n/a	0.0 ± 0.0 a	0.00 ± 0.00 a	7.5 ± 1.1 a
Sugarcane – Sugarcane	1.3 ± 0.2 b	9.6 ± 0.7 b	25.5 ± 3.3 c	0.21 ± 0.38 c	34.3 ± 2.4 c
Sugarcane – Johnsongrass	1.1 ± 0.2 b	8.3 ± 0.5 b	10.5 ± 1.7 b	0.19 ± 0.01 c	20.9 ± 2.4 b
Sugarcane – Columbus	1.0 ± 0.2 b	7.2 ± 0.9 b	10.0 ± 1.3 b	0.17 ± 0.03 c	20.6 ± 1.7 b
	df=4,35; *F*=10.26; *P* < 0.0001	df=4,35; *F*=17.25; *P* < 0.0001	df=4,35; *F*=27.96; *P* < 0.0001	df=4, 35; *F*=13.01; *P* < 0.0001	df=4, 35; F=29.68; *P* < 0.0001

Note: Data are Means ± SE. Statistical significance based on One Way ANOVA. The value with n/a denotes: not available because aphid failed to reproduce (therefore no pre-reproductive period). Values in the same column followed by the same letters are not significantly different at P < 0.05 according to DIFF statement in the LSMEANS.

Table 3. Life table parameters of sugarcane aphids transferred from Columbus grass (CoSCA) to five host plants

Host transfer type	Number of nymphs per day	Pre-reproductive period (d)	Reproductive period (Rp)	Intrinsic rate of increase (r_m)	Longevity (L)
Columbus – Sus. Sorghum	3.7 ± 0.3 a	5.5 ± 0.1 b	17.9 ± 1.4 a	0.43 ± 0.01 c	31.7 ± 1.7 a
Columbus – Res. Sorghum	1.3 ± 0.1 b	6.4 ± 0.4 b	18.6 ± 2.3 a	0.27 ± 0.02 b	28.9 ± 1.6 a
Columbus – Sugarcane	0.4 ± 0.1 c	11.4 ± 2.1 a	7.5 ± 2.1 b	0.03 ± 0.03 a	19.5 ± 2.0 b
Columbus – Johnsongrass	3.5 ± 0.3 a	5.4 ± 0.2 b	15.8 ± 2.4 a	0.37 ± 0.01 c	29.5 ± 0.9 a
Columbus – Columbus	3.4 ± 0.4 a	5.0 ± 0.2 b	21.6 ± 2.2 a	0.41 ± 0.02 c	33.0 ± 1.6 a
	df= 4,28; *F*=43.38; *P*<0.0001	df= 4,35; *F*=7.74; *P*=0.0001	df=4,28; *F*=10.68; *P*<0.0001	df=4,35; *F*=61.8; *P*<0.0001	df=4,35; *F*=10.95; *P*<0.0001

Note: Data are Means ± SE. Statistical significance are based on One Way ANOVA. The value with n/a denotes: not available because aphid failed to reproduce (therefore no pre-reproductive period). Values in the same column followed by the same letters are not significantly different at P < 0.05 according to DIFF statement in the LSMEANS.

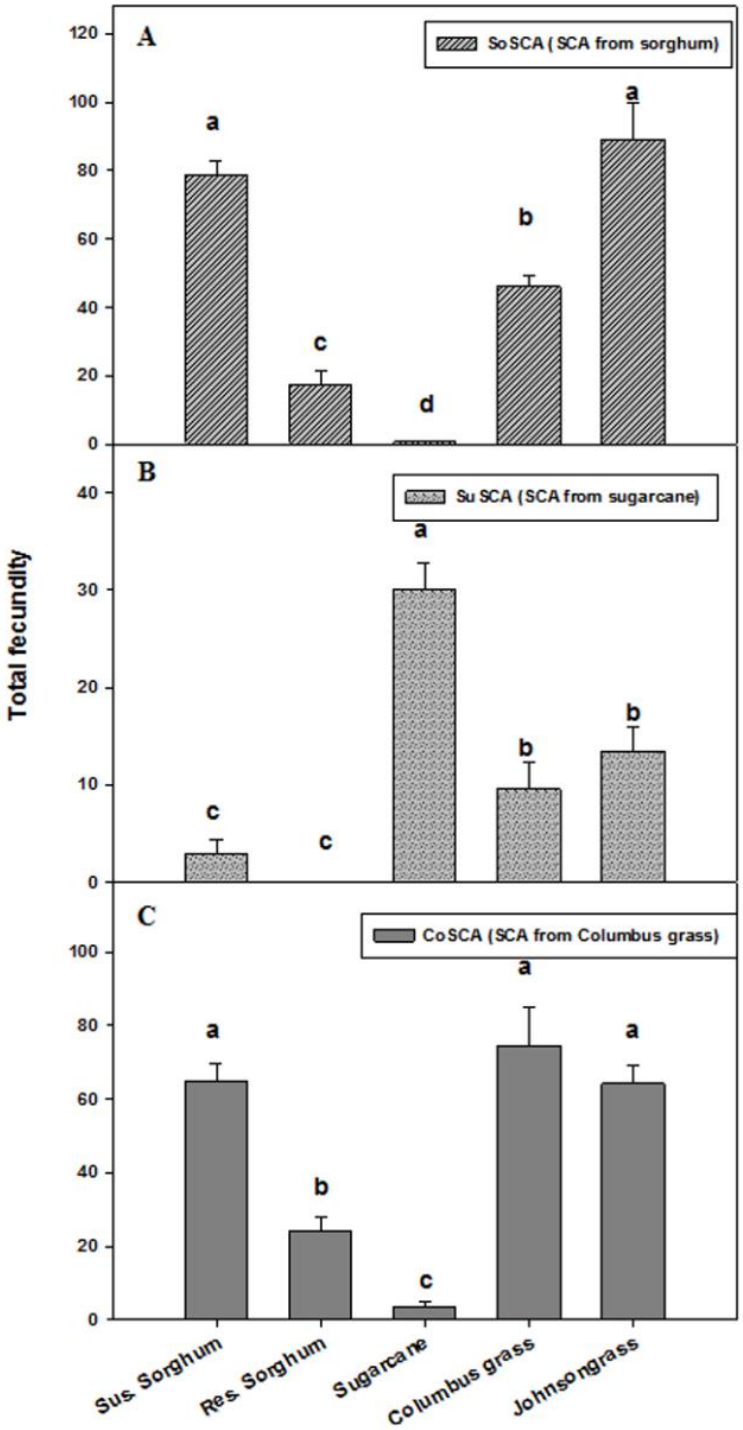

Fig. 1. Total fecundity (Mean ± SE) of aphids from sorghum (Fig 1A, SoSCA), sugarcane (Fig 1B, SuSCA), and Columbus grass (Fig 1C, CoSCA) when transferred to resistant

sorghum (Res.), susceptible sorghum (Sus.), sugarcane, Columbus grass and Johnsongrass. For each aphid, means (top of columns) with the same lowercase letters are not significantly different at $P > 0.05$. Differences among host plants were highly significant for the average number of nymphs per female of SoSCA (df = 4, 28; F = 58.2; $P < 0.0001$), SuSCA (df = 4, 25; F = 30.06; $P < 0.0001$), and CoSCA (df = 4, 35; F =28.01; $P < 0.0001$).

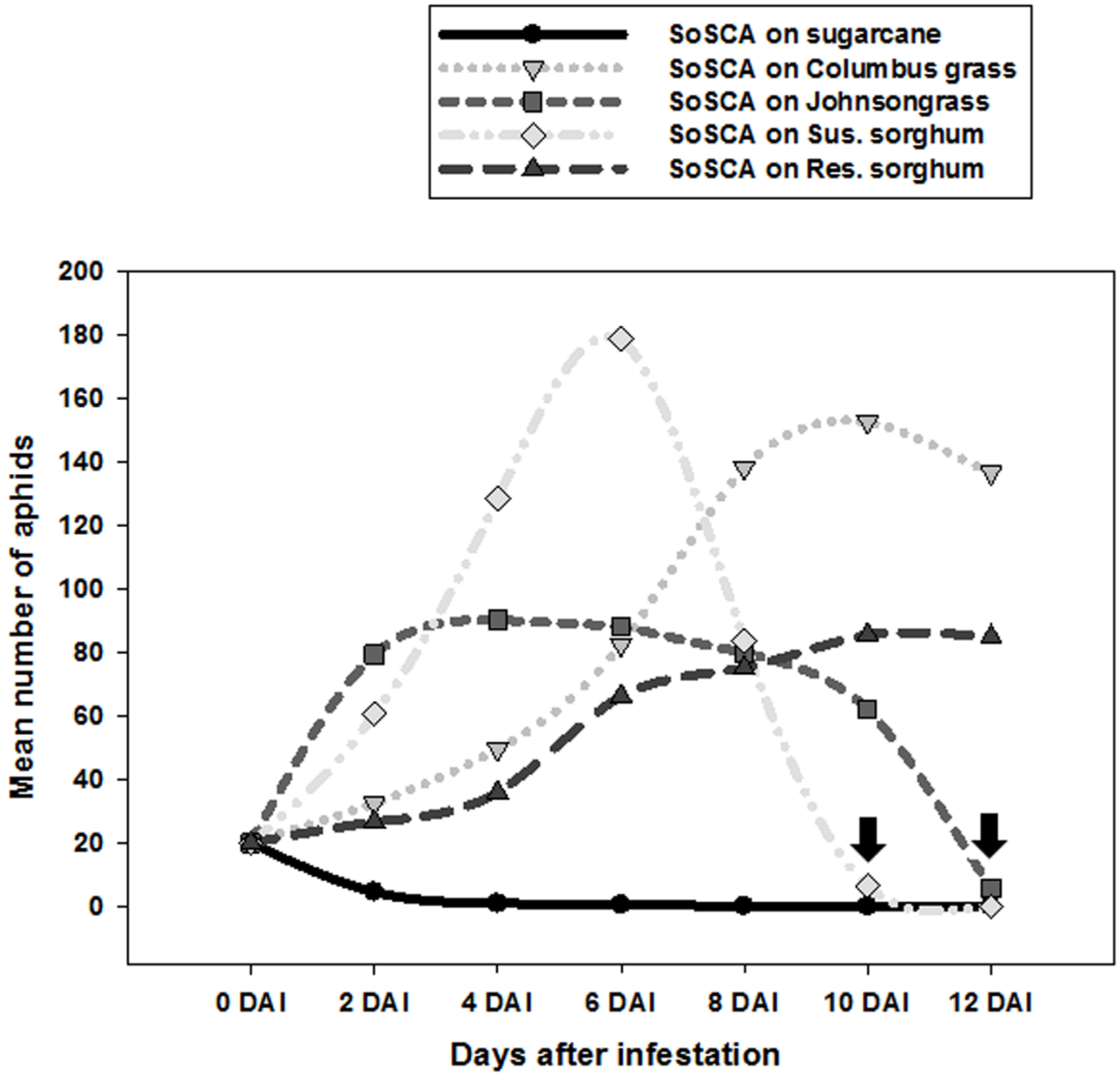

Fig 2. Mean number of SoSCA aphids (aphids originally collected from and maintained on sorghum) during a 12-day time period after transfer to sugarcane, Columbus grass, Johnsongrass, and resistant and susceptible sorghum. Down arrow indicates that the plants (susceptible sorghum and Johnsongrass) were almost dead with a damage rating >8.

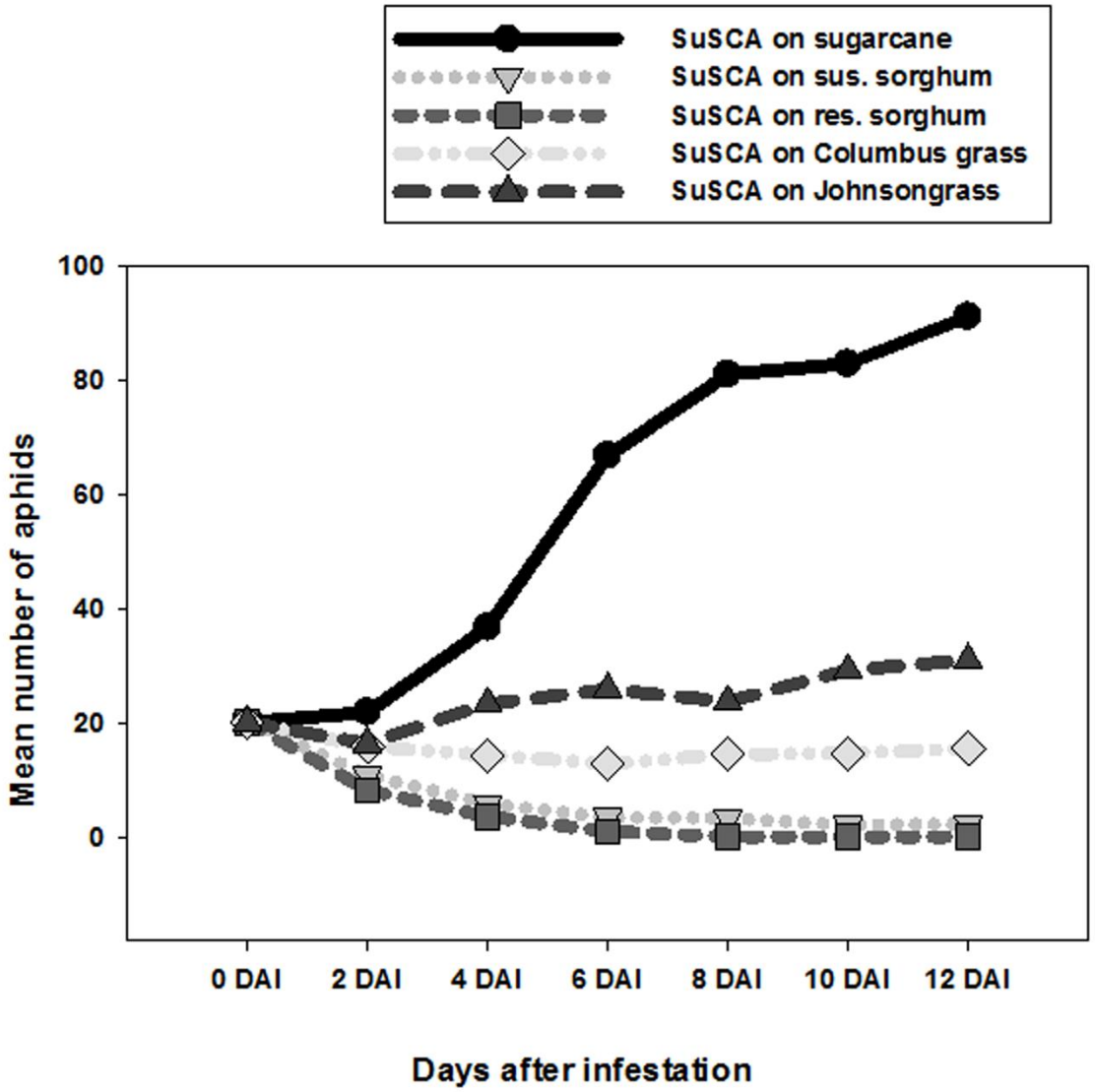

Fig 3. Mean number of SuSCA aphids (aphids originally collected from and maintained on sugarcane) during a 12-day time period after transfer to sugarcane, Columbus grass, Johnsongrass, and resistant and susceptible sorghum.

CHAPTER VI

CONCLUSIONS

Relatively few varieties containing sugarcane aphid (*Melanaphis sacchari* (Zehnter)) resistance genes are available for growers. Since the outbreak of sugarcane aphids in 2013 in sorghum, finding and advancing resistant germplasm has been a priority. Knowledge of physiological response of sorghum to *M. sacchari* feeding will provide baseline information on defense responses and resistance mechanisms of sorghum genotypes. Therefore, the first objective of this research project was to gain insight into the physiological responses of resistant and susceptible sorghum genotypes to sugarcane aphid feeding. Photosynthetic rates and stomatal conductance were measured at different sorghum genotypes and densities using a portable photosynthesis system (LI-COR 6400, Lincoln, NE). Resistant plants when infested with aphids compensated injury by either increasing or maintaining photosynthetic rate and stomatal conductance. A/Ci curves over time showed that infested resistant plants had delays in photosynthetic senescence, whereas susceptible plants displayed accelerated photosynthetic senescence. Differences between physiological responses of infested susceptible and resistant genotypes imply that resistant sorghum plants can tolerate some impacts of *M. sacchari* feeding injury.

The second objective was to identify sources of *M. sacchari* resistance in sorghum by using conventional screening methods. Resistant sorghum genotypes identified in this study will hasten the development of commercially suitable sorghum varieties for sorghum growers in the Southern U.S. Additionally, the mechanism(s) of resistance were determined for evaluated sorghum genotypes that were categorized as resistant. Free-choice and no-choice tests were conducted to explore the categories of resistance (Tolerance, Antixenosis, and Antibiosis). Levels of resistance were compared with the known resistant 'TX 2783' and the susceptible 'KS 585', and four genotypes (AG1201, AG1301, W844-E, and DKS 37-07) were identified expressing high levels of all three categories of resistance. Other genotypes with moderate levels of tolerance and antibiosis could be interesting choices for management of the pest. Tolerant plants are known to sustain lower selection pressure to insect attack, and yield is less affected by infestations, however, tolerance to sugarcane aphids may result in large amounts of honeydew and sooty mold infestations which hinder harvesting. For this aphid pest, it could be more advantageous to grow cultivars that have a combination of antibiosis effects that limit population growth and tolerance traits that limit plant damage.

Genetic diversity has been examined worldwide and in the Americas for the sugarcane aphid. Nibouche et al. (2018) reported two multilocus lineages (MLL-D and MLL-F) in the United States and suggested occurrence of host-associated lineages/genotypes. An objective of this dissertation was to quantify phenotypic behaviors (host suitability as measured through life table statistics) among sugarcane aphid clones collected from different hosts. Diverse groups of sugarcane aphid clones that colonized sorghum (SoSCA), sugarcane (SuSCA), and Columbus grass (CoSCA)

were collected and host suitability was examined when clones were introduced to five different plants including, sugarcane, Columbus grass, Johnsongrass, and resistant and susceptible grain sorghum. The survivorship and reproduction of the sugarcane aphid collected from and maintained on sugarcane was significantly higher when offered sugarcane (>85%) as compared to other hosts and in contrast, there was negligible survival and reproduction when other aphid clones (collected from sorghum and Columbus grass, and maintained on respective hosts) were offered sugarcane as host. Offering new host plants revealed that the other two aphid clones from sorghum and Columbus grass were relatively similar in survival and reproduction, and are likely similar in terms of host plant selection. Separate genotyping revealed that sugarcane collected aphid clones were a different genotype and belong to multilocus lineage MLL-D as compared to sorghum and Columbus grass collected clones which belonged to MLL-F. Genotyping supports the findings of Nibouche et al. (2018) which indicated two genotypes (MLL-D and MLL-D) of sugarcane aphid prevailing in the U.S. The result my research further suggests that these aphid genotypes are likely host-specific, i.e. MLL-F (sugarcane aphid collected from sorghum or Columbus grass) exhibit negligible reproduction when provided sugarcane as a host, but do well in the same hosts (sorghum and Columbus grass) and MLL-D (sugarcane aphid collected from sugarcane) exhibit negligible reproduction on sorghum, but do well on sugarcane. Additional research still needs to be undertaken to examine sugarcane aphids collected from different hosts and locations to fully describe host specificity of local and regional populations.

The importance of host-plant resistance as a foundation of IPM cannot be over emphasized as it is compatible with other multiple management strategies (Biological

Control, Pesticides, Cultural Control, etc.). Several sorghum genotypes studied in chapter IV were observed to have resistance causing low fecundity and population growth. By delaying population growth of the sugarcane aphid via antibiotic or even antixenotic resistance, natural enemies are potentially better able to limit aphid population increases and plant damage. Most of the resistant sorghum genotypes studied here have already been released as varieties offering varying degree of antibiosis, antixenosis and tolerance that will benefit sorghum producers as a foundational tool in the IPM arsenal. Observed antibiotic resistance to sugarcane aphids is most likely based on toxic allelochemicals, and these compounds and the genes controlling their expression need to be identified in future studies. Photosynthetic responses of sorghum genotypes to sugarcane aphid feeding have added to our understanding of the underlying mechanisms of aphid-sorghum interactions. Knowledge of the physiological alterations occurring in sorghum leaves infested by sugarcane aphid may be leveraged into the development of new resistant cultivars. Identification of resistance genotypes as well as knowledge of mechanisms and physiological responses due to aphid feeding can provide important information to sorghum breeders who are looking for more durable sources of resistance.

APPENDICES

EVALUATION OF SORGHUM GENOTYPES FOR RESISTANCE TO THE SUGARCANE APHID UNDER FIELD CONDITIONS

(2016/2017 sugarcane aphid hybrid evaluation, Cimarron Valley Experiment Station, Oklahoma State University Experiment Station)

Methods: Field trials were conducted in 2016 and 2017 to confirm greenhouse evaluations (Chapter IV) where several sorghum genotypes provided by seed companies expressed resistance to the sugarcane aphid.

2016: Twenty different sorghum genotypes (KS 585, GW1456, 12GS9041, 12GS9023, DKS3888, 96275, GW7431, H130373, 95207, GW1489, W844-E, 12GS9017, TX2783, OL2042, 97157, G1213, 12GS9012, DKS-3707, W7051, 97157) were planted in May 2016 in a dryland replicated field trial at the Cimarron Valley Experiment Station, Perkins, OK (plot size: 12 feet * 25 feet). Each genotype had four replications. These genotypes were screened for resistance in a greenhouse at seedling stage as explained in chapter IV. Ten plants from each plot were selected randomly and observed for aphid infestations and damage. Ratings were recorded on 1-9 scale (1= no damage, 2=1-5%, 3= 6-20%, 4= 21-35%, 5=36-50%, 6=51-65%, 7=66-80%, 8=81-95%, 9=96-100%) similar to Sharma et al. (2014). Data for damage ratings were analyzed using the MIXED Procedure of SAS (SAS Institute 2010).

2017: The ten commercial hybrids that were previously shown to have resistance to the sugarcane aphid (SP 73B12 (Sorghum Partners), SP 7715 (Sorghum Partners), BH 3616 (B&H Genetics), BH 4100 (B&H Genetics), Golden Acres 3960B, Warner W-7051, Warner W-844E, and DeKalb 37-07) along with two known susceptible (KS 585 and BH 3822) were planted in same experiment station as above in Perkins to identify resistance. Plots were planted on 30" centers and 40' in length in a complete randomized design with each entry replicated four times. The plots were sampled only one time during the season due to the late appearance of sugarcane aphid. SCA densities and damage ratings were taken on the 30th of August 2017. Sugarcane aphid densities were counted on number of aphids per leaf on one bottom leaf and one upper leaf on 5 plants in each of the center two rows of each plot, for a total of 10 plants (20 leaves) per plot. Leaf damage from SCA feeding was assessed by using a 1-9 scale as mentioned above. Plots were harvested by small plot combine (Oklahoma State University, Cropping Systems Unit) where yield per acre, test weight, and moisture content were recorded. Data for sugarcane aphid numbers, damage ratings were analyzed using the MIXED Procedure of SAS at α=0.05. Yield data components were analyzed using ANOVA, one-way analysis at α=0.05.

Results.

2016-field experiment: Sugarcane aphids were first detected when sorghum was in the late vegetative stage. Heavy rainfall occurred on July 14 and 28, and August 25 which kept SCA population below economic thresholds. SCA numbers in the same plots were not consistent, only some plants in the same plots were severely infested. Significantly higher damage ratings (>3) were observed in KS 585 (known susceptible), GW1456,

12GS9041, and 12GS9023 (Table 1). Because of the late arrival of aphids, inconsistent damage, and lodging of plants in some plots (Figure 1), grain yields of genotypes were not recorded and reported.

2017-field experiment: Yellow sugarcane aphids were first detected in the plots on the 20th of June at very low levels (<3 per leaf on the very bottom leaves)**.** Sugarcane aphids were first detected on July 13 when the sorghum was in the 6 leaf stage. Some heavy rains occurred between July 13 and August 1 where we believe SCA were kept below threshold of 50 aphids per leaf. On 30 August, the number of aphids per upper and lower leaf and damage ratings were taken resulting in the data presented in Table 1. The only two hybrids with significantly higher damage ratings in the trial were the susceptibles BH 3822 (damage rating = 3.5) and KS 585 (damage rating = 5.1), and they also had the highest sugarcane aphids surpassing a thousand and six thousand aphids per leaf, respectively (Table 2). The late infestation of sugarcane aphids and damage ratings below 2.0 for the ten resistant hybrids were unlikely to be a factor affecting yield loss (Table 3.)

Conclusions. Late infestations and rains prevented sugarcane aphids from reaching economically threatening densities at the Perkins site.

Acknowledgement. This project was supported by a grant from the USDA-ARS titled "Area-wide pest management of the invasive sugarcane aphid in grain sorghum." Thanks to the seed companies for providing seed for this trial. Also, thanks to Dr. Josh Lofton and the Oklahoma State University Cropping Systems team for machine harvesting the plots and providing yield data.

Table 1. Sorghum entries planted with seed treatment and evaluated for phenotypic damage from sugarcane aphid, *M. sacchari*, at Perkins (2016), using 1-9 scale.

Sorghum entries	Damage rating ± SE	Sorghum entries	Damage rating ± SE
KS 585	4.08 ± 0.69 a	W844-E	1.87 ± 0.16 c-e
GW1456	3.8 ± 0.73 a	12GS9017	1.7 ± 0.16 c-e
12GS9041	3.52 ± 0.76 ab	TX2783	1.7 ± 0.12 c-e
12GS9023	3 ± 0.89 a-c	OL2042	1.67 ± 0.21 c-e
DKS3888	2.95 ± 0.43 a-c	97157	1.5 ± 0.8 c-e
96275	2.67 ± 0.57 a-d	G1213	1.45 ± 0.16 de
GW7431	2.35 ± 0.46 a-d	12GS9012	1.3 ± 0.12 de
H130373	2.02 ± 0.15 c-e	DKS-3707	1.6 ± 0.08 e
95207	1.9 ± 0.18 c-e	W7051	1.18 ± 0.06 e
GW1489	1.9 ± 0.35 c-e	95157	1.5 ± 0.8 e

Values in the same column followed by the same letters are not significantly different at $P < 0.05$ according to DIFF statement in the LSMEANS.

Table 2. Sorghum entries planted with seed treatment and evaluated for phenotypic damage from sugarcane aphid, *M. sacchari*, at Perkins (2017), using 1-9 scale and mean number of aphids collected from lower and upper leaves.

Plot Entry	Cultivar	Maturity Rating	Damage ratings	Mean number of aphids/leaf
1	AG1201	Early	1.8 ± 0.2 c	359.1 ± 289.4 bc
2	BH 3616	Med	1.6 ± 0.2 c	276.7 ± 266.4 bc
3	BH 4100	Med	1.4 ± 0.1 c	5.6 ± 1.9 c
4	BH 3822	Med	3.5 ± 1.1 b	1,674.4 ± 1142.7 b
5	GA 3960B	Med	1.5 ± 0.2 c	4.2 ± 1.7 c
6	SP 73B12	Med-full	1.2 ± 0.1 c	3.9 ± 1.2 c
7	KS 585	Med	5.1 ± 0.9 a	6,433.1 ± 4292.8 a
8	AG 1301	Med-full	1.8 ± 0.6 c	330.4 ± 201.4 bc
9	SP 7715	Med-full	1.2 ± 0.2 c	431.4 ± 425.9 bc
10	W-7051	Med-full	1.1 ± 0.1 c	5.2 ± 3.9 c
11	W-844E	Med-full	1.4 ± 0.3 c	6.7 ± 4.1 c
12	DKS 37-07	Med-early	1.9 ± 0.4 c	589.4 ± 272.5 bc

Values in the same column followed by the same letters are not significantly different at $P < 0.05$ according to DIFF statement in the LSMEANS.

Table 3. Yield data for sorghum hybrids planted and evaluated for phenotypic damage from sugarcane aphid, *M. sacchari*, at Perkins, OK 2017.

Hybrid	Yield		% Moisture	Test Wt. lbs/bu
	Lbs/ac	Bu/ac		
AG1201	2605	47	9.6	56.0
BH 3616	3008	54	9.0	52.7
BH 4100	3420	61	9.7	59.5
BH 3822	2728	49	10.5	58.7
GA 3960B	2928	52	9.5	56.3
SP 73B12	2339	42	11.4	58.1
KS 585	2644	47	10.8	56.1
AG 1301	2635	47	9.4	60.7
SP 7715	2607	47	9.5	58.7
W-7051	2642	47	9.4	59.8
W-844E	2974	53	9.9	58.2
DKS 37-07	3022	54	9.7	57.5
Average	2796	50	9.9	57.7

Figure 1. Lodging of sorghum plants in some plots due to heavy rain in 2016-field experiment.

Figure 2. Sugarcane aphids on underside of leaf (clicked from 2016 plots).

Figure 3. Sorghum field (2017)

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