

6-29-2018

Impact of Temperature, Plant Species, and Sorghum Cultivar on the Population Dynamics of *Melanaphis sacchari*

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**IMPACT OF TEMPERATURE, PLANT SPECIES, AND SORGHUM CULTIVAR ON THE
POPULATION DYNAMICS OF *Melanaphis sacchari***

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Entomology

by

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August 2018

ACKNOWLEDGMENTS

I express my sincere gratitude to my major professor, Dr. Jeffrey A. Davis, whom received me years ago, and since then has been a source of guidance and encouragement. Dr. Davis guided me since the planning of this project and always provided critical review of my work. Working with Dr. Davis inspires me to always be open to learning and achievement. My professional and personal growth under Dr. Davis' supervision is immeasurable, and I will never find enough words to express my gratitude towards him.

I am also grateful to my other committee members, Drs. Michael Stout, Rodrigo Diaz, Thomas Reagan, and Dean's representative Dr. Yi-jun Xu for serving on my committee and sharing valuable suggestions throughout the years.

Special thanks to Dr. Gaomin Wang, from the Department of Experimental Statistics. Dr. Wang helped me to select the best analyze for each experiment in this project. She also reviewed each analyze thoroughly and always made sure I had a complete understanding of each test used.

From the Soybean Laboratory, I am thankful to Jie Chen for the help with the life-table studies, Sunil Paudel and John Dryburgh for helping me with field trials, and all the other students for the friendship. I also thank Jeffrey Murray and Xuan Chen for the collaboration and friendship throughout this journey.

I also acknowledge the financial support from the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES). This project was also partially funded by the Louisiana Soybean and Grain Promotion Board and the Louisiana State University Agricultural Center.

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ABSTRACT

Sugarcane aphid, *Melanaphis sacchari* Zehner, is now widely established in sorghum, *Sorghum bicolor* L., production areas of the United States and is an important economic pest. However, detailed studies of temperature, host range and plant resistance effects on the biology and population parameters of the *M. sacchari* biotype responsible for the sorghum outbreak in the United States have not been performed previously. Therefore, the present study aimed to investigate: 1) How temperatures affect *M. sacchari* 2) *M. sacchari* interaction with host plants 3) *M. sacchari* interaction with sorghum genotypes. The response of *M. sacchari* to six different constant temperatures (15, 20, 25, 30, 32, and 35°C) on sorghum plants was evaluated in the laboratory. Population dynamics and feeding behavior of *M. sacchari* on six host plants were determined through life table studies and by using the electrical penetration graph (EPG) technique. Twelve sorghum genotypes were screened in laboratory bioassays for potential *M. sacchari* resistance. From those, seven genotypes were selected and tested in greenhouse and field trials, and feeding behavior was characterized by EPG technique. Population parameters, together with high minimum and maximum thermal thresholds, indicate that *M. sacchari* is an aphid species more adapted to higher temperatures than other species. Sorghum and Johnsongrass are highly suitable host plants, and sieve element pathway phase is important in host plant selection as well as phloem based factors. Sorghum genotypes PI524770, PI564163, and PI643515 were consistently resistant to *M. sacchari*. These findings will provide a more robust basis for integrated pest management of *M. sacchari* on sorghum.

CHAPTER 1. GENERAL INTRODUCTION

The sugarcane aphid, *Melanaphis sacchari* Zehntner, has worldwide distribution and has been present in the United States on sugarcane since 1896 (Mead 1978). In the United States, losses in sugarcane to this insect are caused mainly by the transmission of sugarcane yellow leaf virus (Hall and Bennet 1994, Schenck and Lehrer 2000, Paray et al. 2011). In 2013, this aphid host switched to sorghum with infestations reported in Texas, Louisiana, Oklahoma, and Mississippi (Villanueva et al. 2014, Bowling 2016). In 2014 and 2015, estimated of losses due to *M. sacchari* infestation in sorghum ranged from \$1 million to as high as \$35 million (Brewer and Gordy 2016). Heavy infestations required up to four insecticide applications to control *M. sacchari* (Brewer et al. 2017). Cost of control alone reached \$10.5 million in Texas and more than \$10 million in Louisiana and Mississippi (Brewer and Gordy 2016).

Outbreaks of *M. sacchari* occurring on sorghum throughout the United States are associated with one single genotype. Harris-Shultz et al. (2017) found the same genotype in 17 locations in seven states and Puerto Rico. Nibouche et al. (2018) found that one *M. sacchari* genotype represented 90% of the specimens collected in nine states in the United States. Nibouche et al. (2018) also observed that this *M. sacchari* genotype was genetically distinct from genotypes previously collected in Louisiana (Nibouche et al. 2014). Nibouche et al. (2018) hypothesized that this is a foreign introduction into the Americas either from Africa or Asia.

Studies into the management of *M. sacchari* in sorghum include insecticide-treated seeds (Jones et al. 2015), scouting, foliar applications of sulfaxoflor or flupyradifurone, use of plant natural extracts (Yadav et al. 2016), biological control (Colares et al. 2015, Colares et al. 2017),

and host plant resistance (Teetes et al. 1995, Sharma et al. 2013, Sharma et al. 2014, Armstrong et al. 2015, Brewer et al. 2017, Szczepaniec 2018). The availability of multiple control strategies in this system provides an opportunity for development of an integrated pest management (IPM) program but a better understanding of *M. sacchari* life traits are needed to fully implement it. Additionally, this *M. sacchari* clone shows extensive distribution and high frequency, and thus, it is expected to show differential fitness and behavioral responses across distinct environmental conditions (Vorburger et al. 2003, Chen et al. 2013, Harrison and Mondor 2011).

Melanaphis sacchari is an oligophagous species observed on 17 plants in the family Poaceae and in two plants genera in the family Araceae (Denmark 1988, Blackman and Eastop 2000, Singh et al. 2004). Despite reports of *M. sacchari* feeding on numerous plants, the relative suitability of these hosts, the degree to which they are utilized, and the effects on population dynamics are not well understood. Colonizing aphids are those species that settle and reproduce on the host in question (Wosula et al. 2013). Since most of the reports of *M. sacchari* on different plants are observational data, it cannot be assumed that colonization will take place on those plants.

Understanding how population dynamics are affected by temperature is also important for management of *M. sacchari*. On sorghum, *M. sacchari* is found in a wide range of climates, from 0 to 35°C (Singh et al. 2004). *Melanaphis sacchari* life cycle can be completed as little as 4.3 days or be as long as 37 days (Singh et al. 2014), and its optimum temperature for development ranges between 20 and 25°C (Setokuchi 1973).

Decisions such as when to initiate and apply integrated pest management strategies are dependent upon population growth during the growing season, which in turn depends on how

temperature affects the pest (Metcalf and Luckman 1994, Thindwa and Teetes 1994). For example, spraying and release of natural enemies can be adjusted according to the population growth of the pest (Kingsolver 1989, Bernal and Gonzáles 1997)

Parallel with the understanding of pest's biology and ecology, investigation of potential resistance sources is important to improve IPM. Sorghum genotypes resistant to greenbug, *Schizaphis graminum*, have been identified and are used extensively in sorghum. Shortly after shifting from wheat fields to sorghum fields, over 100,000 ha of the crop was destroyed and millions of hectares had to be sprayed for greenbug, and by 1981 greenbug was ranked as the second most damaging insect pest of sorghum in the United States (Michels and Burd 2007). After the first outbreaks, antibiosis and tolerance mechanisms of resistance were identified (Koch et al. 2014), and sorghum lines with resistance to greenbug were used as parental lines in commercial sorghum hybrid development (Royer et al. 2015). The research on the development of resistant sorghum hybrids insured the economic viability of sorghum production in the United States (Michels and Burd 2007).

The effect of temperature and fitness and behavioural responses of this *M. sacchari* genotype collected from sorghum and associated with the current outbreak in the United States has not been studied. Plant Introduction (PI) genotypes expressing different levels of resistance to greenbug may be resistant to *M. sacchari* as well. These lines can provide parental lines for development of *M. sacchari* resistant sorghum hybrids. This project characterizes levels of resistance to *M. sacchari* in sorghum PIs in laboratory, greenhouse and field experiments, and determines specific host plant resistance mechanisms using the electrical penetration graph (EPG) technique. This study also helps to increase our understanding of *M. sacchari* population

dynamics on potential hosts of *M. sacchari* found in the Louisiana agroecoscape, and understand the feeding behavior of *M. sacchari* on these host plants using the EPG technique. Development of *M. sacchari* population dynamics on sorghum under six constant temperatures were used to estimate upper and lower developmental thresholds and optimum developmental temperature. These findings provided information on aphid scouting time, landscape distribution, and resistance sources that can help improve management of this pest.

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CHAPTER 2. LITERATURE REVIEW

2.1. Sorghum

Grain sorghum, *Sorghum bicolor* (L.) Moench, is one of the most important cereal crops grown in the United States. In 2016, sorghum was planted on 2.7 million hectares, and 480,261 million bushels were harvested (NASS, USDA 2017). The 'Sorghum Belt' runs from South Dakota to Southern Texas, and the top five sorghum-producing states in 2017 were Kansas, Texas, Oklahoma, Colorado, and Arkansas (NASS, USDA 2018). Sorghum is used for biofuel and for animal and human consumption. Approximately one-third of the United States sorghum crop is used for renewable fuel production, 35% is used to produce animal feed, and 42% is exported (U.S. Grain Council 2016).

Sorghum is attacked by native and invasive pests which can significantly reduce yield. Insects in the Order Coleoptera such as billbug, *Sphenophorus callosus* Olivier (Coleoptera: Curculionidae), sugarcane beetle, *Euethola rugiceps* LeConte (Coleoptera: Scarabeidae), southern corn rootworm, *Diabrotica undecimpunctata* Howardi Barber (Coleoptera: Chrysomelidae), southern potato wireworm, *Conoderus falli* Lane (Coleoptera: Elateridae), and tobacco wireworm, *Conoderus vespertinus* Fabricius (Coleoptera: Elateridae) cause injury to sorghum leaves and roots, resulting in yield losses (van Rensburg and Hamburg 1975, Johnson et al. 1979, Buntin 2009). In the Order Lepidoptera, black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae), granulate cutworm, *Feltia subterranean* Fabricius (Lepidoptera: Noctuidae), corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), European corn borer, *Ostrinia nubilalis* Hübner (Crambidae: Lepidoptera), fall armyworm, *Spodoptera*

frugiperda JE Smith (Lepidoptera: Noctuidae), lesser cornstalk borer, *Elasmopalpus lignosellus* Zeller (Lepidoptera: Pyralidae), and stalk borer, *Papaipema nebris* Guenée (Lepidoptera: Noctuidae) can also reduce yields (van Rensburg and Hamburg 1975, Johnson et al. 1979, Buntin 2009).

The Order Hemiptera contains small insects that suck the sap of the plant. Chinch bug, *Blissus leucopterus leucopterus* Say (Hemiptera: Lygaeidae), and the aphids corn root aphid, *Anuraphis maidiradicis* Forbes (Hemiptera: Aphididae), corn leaf aphid, *Rhopalosiphum maidis* Fitch (Hemiptera: Aphididae), greenbug, *Schizaphis graminum* Rondani (Hemiptera: Aphididae), yellow sugarcane aphid, (*Sipha flava* Forbes (Hemiptera: Aphididae) and sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae) are the primary hemipterans attacking sorghum (van Rensburg and Hamburg 1975, Johnson et al. 1979, Buntin 2009). Aphids are important pests as infestations reduce farm revenue due to yield losses, control costs, and increased input costs to maintain farm machinery (van Rensburg and Hamburg 1975, Johnson et al. 1979, Buntin 2009).

2.2. General aphid biology

Aphids are in the Superfamily Aphidoidea. They are small, soft-bodied insects with high reproductive capacity. Aphids are polymorphic and can be apterous (wingless) or alate (winged) depending on their function and environmental conditions (Blackman and Eastop 2000, Dixon 2012, Williams and Dixon 2007). Aphids have a worldwide distribution, with more than 4,000

known species, all of them being herbivorous and many species are important agricultural pests (Dixon 2012, Williams and Dixon 2007).

Aphids can distribute the functions of reproduction, dispersal, population growth, and overwintering through generations, and to different host plants. For instance, if resources become limited in a colony, females will produce alate individuals for dispersion to other sites (Dixon 2012). Aphidoidea contain species which can be plant host alternating (heteroecious) or nonhost alternating (monoecious) (Williams and Dixon 2007).

Heteroecious species live on a primary plant host during winter and migrate to a secondary plant host in spring. When day length shortens and temperatures fall, heteroecious species migrate back to their primary host (Williams and Dixon 2007). Aphids with heteroecious life-cycles are normally holocyclic, producing sexual morphs (gynoparae and males) on the secondary host that will return to the primary host to mate and oviposit (Williams and Dixon 2007). Cold tolerant eggs (can tolerate temperatures up to -40 °C), such as *Rhopalosiphum insertum* Walk, *Acyrtosiphon svalbardicum* Heikinheimo and *Acyrtosiphon brevicorne* Hille Ris, will survive adverse winter conditions and hatch in spring, giving rise to the fundatrix, the most fecund aphid morph (James and Luff 1982, Strathdee et al. 1995, Williams and Dixon 2007). On the primary host (usually an arborous plant), the fundatrix will produce through thelytoky parthogenesis (parthenogenesis that only produces females), numerous offspring called spring migrants. These migrants will leave the primary host and select a secondary host (an abundant herbaceous plant) where several parthenogenic generations will develop during the summer (Dixon 2012, Williams and Dixon 2007). Because of high temperatures and abundant resources,

aphid populations usually peak in summer (Dixon and Kindlmann 1990, Dixon 2012, Williams and Dixon 2007).

Monoecious aphids (species that do not switch host plants), such as the pea aphid, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae), and the English grain aphid, *Sitobion avenae* Fabricius (Hemiptera: Aphididae), can be important crop pests as well. Monoecious species may be holocyclic (producing sexual morphs), or anholocyclic. When monoecious aphids are anholocyclic, they do not produce sexual morphs, and they overwinter as parthenogenic nymphs or adults (Dixon 2012, Williams and Dixon 2007). Some aphid species can be both holocyclic and anholocyclic, depending on the climate or the availability of primary hosts. For example, *Myzus persicae* Sulzer (green peach aphid), is mostly anholocyclic in tropical regions and holocyclic in temperate areas (Dixon 2012, Williams et al. 2000, Williams and Dixon 2007).

Due to their feeding mechanism and behavior, aphids can cause direct and indirect injury (Peters et al. 1991, Quisenberry and Ni 2007). Injury is defined as any physiological stress from insect feeding (Stout 2013, Stout 2014). Direct injury by aphids is due to the removal of plant photosynthates, piercing of the plant foliage that causes deformation, necrosis, or stunting of new leaves (Blackman and Eastop 2000, Kindler and Hammon 1996, Donahue et al. 1998, Breen and Teetes 1990). This feeding often causes weakening and yellowing of the plant (Burd et al. 1993, Jiang and Miles 1993, Blackman and Eastop 2000). Indirect injury arises from sooty mold growth on the leaves, the injection of toxins which reduce photosynthetic capability, and plant viruses (Quisenberry and Ni 2007).

Although high amounts of plant sap are consumed daily by aphids, the high carbon-nitrogen (C: N) ratio in sap usually results in nitrogen deficiency. Amino acids are essential for

aphids not only for protein synthesis, but they are also neurotransmitter precursors, consumed during respiration, and energy source for some types of cells (Douglas and van Emden 2007). However, in general, plant sap does not provide the complete spectrum of amino acids required. As a result, aphids have developed an obligatory symbiont relationship with bacteria that meet the inadequacy of phloem sap. *Buchnera aphidicola* is a gamma-proteobacteria that supplement the aphid diet by secreting essential amino acids and vitamins (Douglas 1998, Douglas and Prosser 1992, Douglas et al. 2001).

In addition, because of the poor nutritional quality of phloem sap, aphids have a differentiated digestive system. Their digestive system includes a filter chamber, a structure that links the initial portion of the foregut (mesentery) with the anterior portion of the hindgut (proctodeal). The plant sap is concentrated in the filter chamber and absorbed by the aphid, while the excess of liquid and sugars present in the ingested sap are moved directly to the final portion of the digestive system and eliminated as carbohydrate-rich droplets (honeydew) (Waterhouse 1957, Cristofolletti et al. 2003). Due to this mechanism of digestion, aphid colonies can produce vast amounts of honeydew which covers leaf surfaces, facilitating the development of fungus. This fungus grows over the leaf and covers it in black sooty mold, reducing photosynthesis, reducing plant fitness (Teddars 1976, Hall 1987, Woodl 1988).

However, the most economically important indirect effect of aphids is virus transmission to plants. Approximately half of the more than 600 known plant viruses are thought to be vectored by aphids (Ng and Perry 2004, Katis et al. 2007, Blackman and Eastop 2000). Aphids are the most efficient virus vector among insects, and indirect damage from virus transmission can

result in yield decline and quality (Harris 1990, Nault 1997, Pirone and Perry 2002, Ng and Perry 2004).

Among the three aphid species mentioned above that attack sorghum, *R. maidis* and *M. sacchari* transmit sugarcane yellow leaf virus (ScYLV) (El Sayed et al. 2015). Worldwide, *M. sacchari* is an efficient virus vector of sugarcane mosaic virus, millet red leaf virus and sugarcane yellow leaf (Bhargava et al. 1971, Setokuchi and Muta 1993, Blackman and Eastop 2000, Schenck and Lehrer 2000). In the United States, there is only one report of asymptomatic ScYLV infecting grain sorghum (Wei et al. 2017).

Although sugarcane and sorghum are the preferred hosts of *M. sacchari*, the range of virus transmission by *M. sacchari* is more extensive. Laboratory studies by Schenck and Lehrer (2000) tested ScYLV transmission by viruliferous *M. sacchari* on plants in the genus *Saccharum* and alternative host plants. They observed that all *Saccharum* species were infected with SCYLV. Corn, *Zea mays* L., and rice, *Oryza sativa* L., seedlings had low susceptibility to ScYLV. Sorghum, *Sorghum vulgare* × *S. vulgare* var. *sudanense*, had moderate susceptibility, and wheat, *Triticum aestivum* L., oats, *Avena sativa* L., and barley, *Hordeum vulgare* L., were very susceptible (Schenck and Lehrer 2000). Later, ElSayed (2013) used 10-30 *Melanaphis sacchari* individuals to transmit ScYLV to maize plants and confirmed successful transmission by RT-PCR. *Melanaphis sacchari* is also acknowledged to transmit sugarcane mosaic virus (ScMV) to sorghum in India (Bhargava et al. 1971, Kondaiah and Nayudu 1984) and Japan (Setokuchi and Muta 1993).

2.3. *Melanaphis sacchari* life history traits

2.3.1. Temperature

Melanaphis sacchari is found in a wide range of climates, varying from places where the temperature in winter can be below 0°C to localities with an average of 30/35°C (Singh et al. 2004). As a multivoltine poikilotherm insect, *Melanaphis sacchari*'s life cycle is affected by environmental oscillations. *Melanaphis sacchari* may develop up to 61 generations per year (Chang et al. 1982), and its life cycle can be completed as fast as 4.3 days or be as long as 37 days (Singh et al. 2014). Under 15°C, the aphid takes 10.9 days to become an adult, and at 20°C, 25°C, and 30°C, it takes 7.3, 5.2 and 3.5 days to complete its life cycle, respectively (Setokuchi 1973). In the most recent study, Michaud et al. (2017) observed that *M. sacchari* on sorghum plants took 5 to 6 days to develop at 23°C. Overlapping generations are common and all the development stages can be found in a colony (Singh et al. 2004).

Melanaphis sacchari offspring production is also dependent on environmental conditions. According to Chang et al. (1982), fecundity is higher in summer than in winter, and depending upon the temperature range, the aphid could produce up to 96 nymphs per female (Chang et al. 1982, López and Fernández 2002), resulting in infestations up to 30,000 aphids per plant (van Rensburg 1973).

2.3.2. Host range

Most aphids are specialists, as only 5% of aphid species can be categorized as polyphagous (Blackman and Eastop 2000). For pest management it is important to know the pest status of aphids that are found within a field. Aphids entering a field and landing on a crop can be

colonizers and non-colonizers. Colonizing aphids are those aphid species that settle and reproduce on the host in question (Wosula et al. 2013). However, non-colonizing aphids accept plant species when sap ingestion lasts longer than 10 min (Montllor and Tjallingii 1989, Powell et al. 2006, Pettersson et al. 2007). These accepted plant species can be used as bridging species while the colonized plant is not available (Price and Waldbauer 1994, Davis and Radcliffe 2008). Knowing which plant species can be colonized or be used as bridging species contributes to the planning of the spatial and temporal IPM strategies to avoid or predict massive colonization on a crop field (Price and Waldbauer 1994).

Melanaphis sacchari is a cosmopolitan species known worldwide for its losses on sugarcane, *Saccharum officinarum* L. (Blackman and Eastop 2000) and sorghum, *Sorghum bicolor* (Singh et al. 2004). It is a well-recognized sorghum pest in South America, China, Taiwan, Japan, India, South Africa, and Botswana (Singh et al. 2004). Although *M. sacchari* is often associated only with sugarcane and sorghum, Singh et al. (2004) and Blackman and Eastop (2000) mention *M. sacchari* was observed on more than 15 plants of the Poaceae family. *Melanaphis sacchari* have also been observed on *Arum* and *Caladium* plants of the family Araceae family (Denmark 1988). *Melanaphis sacchari* phloem ingestion under no-choice conditions are reported in wheat, *Triticum aestivum* L., oat, *Avena sativa* L., barley, *Hordeum vulgare* L., and maize, *Zea mays* L. (Schenck and Lehrer 2000, ElSayed 2013).

Since aphid colonization is different of plant exploitation, more research is necessary to elucidate whether sorghum is the most suitable plant host for development and population growth of *M. sacchari*, and which host plants are truly colonized by *M. sacchari*.

2.3.3. Life table studies

A life table is a detailed description of the mortality of a population in the various stages of its development (Carey 1993). Life table studies relate development, survivorship and fecundity, and can be used to obtain population dynamics data (Awmack and Leather 2007). There are two general forms of life table, cohort life table and current life table. The current life table observes the age-specific mortality rates of a non-uniform population for a certain period of time. The cohort life table computes the age-specific survival rates of the population from the moment of birth throughout consecutive ages until death of all individuals in the initial cohort (Corey 1993). Both life tables can be completed or not. On a completed life table, the functions of survival and reproduction are computed each day. On a non-complete (abridged) life-table, the computed intervals are periods of development, such as egg, larval period, reproductive period, post-reproductive period (Carey 1993).

Population dynamics studies, which are part of life table studies, provides excellent assistance on the better understanding of the pest-crop system being studied. Life table data can be used to make more assertive decisions on when to apply pest control (Metcalf and Luckmann 1994). For instance, life tables statistics indicate that green peach aphid had its highest reproductive potential among cereals on winter wheat, with rye, *Secale cereale* L. > barley, *Hordeum vulgare* L., > oats, *Avena sativa* L.) (Davis and Radcliffe 2008). These findings have a great impact on potato, *Solanum tuberosum* L., management, because green peach aphid can successfully colonize barley and rye, giving it the potential to rapidly increase early in the season and subsequently colonize potato (Davis and Radcliffe 2008).

Since life-table studies are particularly sensitive to detect any change, the fundamental assumption underlying its use is that experimental conditions remain constant throughout the life of the aphids being evaluated (Awmack and Leather 2000). Thus they are the perfect tool to test the effect of a specific factor, such as temperature or host plants, on an aphid population.

2.4. *Melanaphis sacchari* on sugarcane

On sugarcane, *M. sacchari* is one of the most abundant pest. Populations of *M. sacchari* concentrates mostly on the undersurface of sugarcane leaves. The colonies produce large amounts of honeydew that accumulates on the surface of the lower leaves, promoting the growth of sooty mold fungi. Because of the fungal accumulation on the leaves surface photosynthesis is reduced, consequently affecting sugarcane yield (Hall and Bennet 1994, Nuessly et al. 2015). In Louisiana, *M. sacchari* population peaks during June and July, and *M. sacchari* was more abundant than *S. flava* on five commercial sugarcane cultivars and on all sampling dates evaluated by Akbar et al. (2011). *Melanaphis sachhari* also transmits the persistent sugarcane yellow leaf virus (ScYLV), a *Polerovirus* of the *Luteoviridae* family (Blackman and Eastop 2000, Schenck and Lehrer 2000, Gonçalves 2005, Paray et al. 2011, ElSayed et al. 2015) to sugarcane that reduce yield.

Sugarcane yellow leaf virus is considered to be the most important viral disease of sugarcane in the United States (Schenck and Lehrer 2000, Grisham et al. 2001, ElSayed et al. 2015). In Florida, 14% of yield losses due to *M. sacchari* infestations were reported on sugarcane (Flynn et al. 2005). Sugar yield losses due to ScYLV in Louisiana were up to 14 % (Grisham et al.

2001) and losses up to 11 % of both stalk weight and sugar yield were reported by Comstock and Miller (2004). McAllister et al. (2008) determined that in Louisiana, ScYLV infection was present in all sugarcane-production areas. Disease progress curves indicated that the greatest temporal increase of virus infection coincided with the initial infestation and increase of *M. sacchari* populations (McAllister et al. 2008).

Melanaphis sacchari infestations on sugarcane are also a major problem in South America. In Brazil, ScYLV has caused epidemics and yield losses of up to 50% on sugarcane, which is the major crop for biofuel production of the country (Vega 1997, Gonçalves, 2005). However, direct yield losses due to *M. sacchari* feeding have not been recorded either in North nor in South America.

2.5. *Melanaphis sacchari* on sorghum

Melanaphis sacchari (Zehntner, 1897) impacts on sorghum have been reported in Africa, Asia, Australia, and parts of Central and South America. Prior to 2013, sugarcane aphid was found infesting only sugarcane in the states of Florida, Louisiana, and Hawaii (Mead 1978, White et al. 2001, Bowling et al. 2016a). However, in 2013 the first information of the aphid feeding on sorghum was reported in Texas. Following the occurrence in Texas, Louisiana, Oklahoma, and Mississippi also observed *M. sacchari* infestation on sorghum (Villanueva et al. 2014, Bowling 2016a).

In 2014, *M. sacchari* tripled its range, reaching 12 sorghum producing states with infestation occurring early season. In 2015, all of the 17 states producing sorghum in the United

States reported *M. sacchari* outbreaks (Villanueva et al. 2014, Kerns et al. 2015, Bowling et al. 2016a). In Louisiana and Texas aphid population growth can reach over 900 aphids per leaf, with yield declines of 60 to 100% (Brewer et al. 2017). The heavy infestations require up to four insecticide applications to control *M. sacchari* on sorghum (Brewer et al. 2017). In the first years of the outbreak, losses incurred due to *M. sacchari* infestation in sorghum ranged from \$1 million in Alabama to as high as \$35 million in Texas where \$10.5 million was spent on *M. sacchari* control alone. In Louisiana and Mississippi, 100% of the sorghum fields were infested, and more than \$10 million was spent to control *M. sacchari* (Brewer and Gordy 2016).

Because of the very low temperatures, further north *M. sacchari* overwinter ability is uncertain. Field observations during the winter found *M. sacchari* on sorghum and Johnsongrass in southern areas (Bowling et al. 2016a), and wind-aided movements are known to carry aphids over long distances (Irwin 1999, Irwin et al. 2000, Isard and Gage 2001, Irwin et al. 2007). Thus, both overwintering capacity and wind assisted migration may be responsible for the rapid colonization of all sorghum growing areas each year.

Direct losses are due to the sap extraction of the enormous colonies developed under the sorghum leaves, removing nutrients and carbohydrates in high amounts (Singh et al. 2004, Villanueva et al. 2014, Brown et al. 2015, Bowling et al. 2016a). Also, the massive sap extraction results in high honeydew excretion and deposition on leaves, which results in sooty mold growth and diminished photosynthetic capacity. Young plants suffer because of the constant feeding, showing signs of chlorosis and necrosis (Singh et al. 2004, Kerns et al. 2015). During the plant reproductive stage (pre-flowering, flowering, and grain development) heavy infestations can cause yield reductions and even plant death. Substantial infestation during grain maturation

causes losses due to decrease in harvest efficiency (Kerns et al. 2015, Bowling et al. 2016a, Brewer et al. 2017).

In Louisiana, a reduction of 27% in yield was reported in sorghum by Kerns et al. (2015). Kerns et al. (2015) also described an increase on costs due to problems in harvesting, such as a 22% reduction on harvesting speed and more than 40 hours of pauses due to machinery breakage. In 2014, the total impact of the sugarcane aphid was estimated to be \$7.6 million in Louisiana alone, with damaging infestations present in 85% of the grain sorghum acreage in the state (Brewer and Gordy 2016). In 2016, the area planted with sorghum was reduced by 21.2 % in comparison with 2015 (NASS, USDA 2017). Total grain sorghum production in 2016 also experienced a 20% decrease in comparison with the previous year (NASS, USDA 2017). In 2017 the sorghum planted area was reduced by 10.5 % in comparison with 2016 (NASS, USDA 2018). With these alarming reports, all sorghum production regions in the United States are at risk. *Melanaphis sacchari*'s high reproduction capacity allows the aphid to spread rapidly across a wide geographic range, and entire sorghum fields have been lost due to the infestation of *M. sacchari* (Brewer et al. 2016, Brewer and Gordy 2016).

2.5.1. New biotype or foreign invader?

Because of the host shift and the rapid spread, there is the concern that an invasion of a sorghum-adapted host-associated genotype from another region could have caused this sudden *M. sacchari* outbreaks on sorghum. Before the 2013 outbreaks, Nibouche et al. (2014), in a population genetics study carried out using 10 microsatellite markers and cytochrome oxidase I (COI) barcoding, showed that *M. sacchari* populations collected worldwide during 2002-2009 could be categorized into five multilocus, or clonal, lineages. Multilocus lineages (MLL) are

clusters of parthenogenetic lineages grouping multilocus genotypes (genotypes differing from each other by stepwise mutations). Each of the five MLL grouped individuals from (A) Africa, (B) Australia, (C) South America, the Caribbean, and the Indian Ocean, and (E) China. Nibouche et al. (2014) classified the genotypes on Hawaii and Louisiana as MLL-D while the genotype found in Puerto Rico as MLL-C.

In post-outbreaks studies, Medina et al. (2017) used AFLP markers to examine the genetic similarity of SCA specimens collected on sorghum, Johnsongrass, and sugarcane in eight states from as many locations within each state as possible. The authors detected 31 *M. sacchari* genotypes and grouped them into three distinct MLL, but the clusters do not correspond with any specific host plant or geographic association (Medina et al. 2017). It is noteworthy that the authors detected three genetically distinct clusters, while Nibouche et al. (2014) identified only one multilocus lineage in the continental United States. Thus the authors suggested that the lack of host-associated differentiation and the absence of geographic clustering do not seem to support the hypothesis of a recent introduction of a sorghum-specialized genotype (Medina et al. 2017).

In a second study, Harris-Shultz et al. (2017) sampled *M. sacchari* on sorghum and Johnsongrass in 17 locations of seven Southern states and Puerto Rico. By genotyping with 52 microsatellite markers, the authors observed a very low genetic diversity, with one predominant biotype across the locations, except a single sample collected from Sinton, TX, which had the predominant genotype as well as another genotype (Harris-Shultz et al. 2017). Here the authors found the same genotype predominant in Louisiana and Puerto Rico, while Nibouche et al. (2014) found different multilocus lineages in these locations. Therefore from Harris-Shultz et al. (2017)

study conclusions, it appears that the invasive *M. sacchari* on sorghum is spreading in the United States on sorghum as primarily one asexual clone.

So far in the United States, the mentioned studies revealed a low level of genetic differentiation among *M. sacchari* populations without a clear geographical or host-associated differentiation. The differences between the studies (i.e., one predominant genotype versus three multilocus lineages) are likely due to different strategies for their genetic analysis. While Medina et al. (2017) genotyped individual specimens of *M. sacchari* collected across the sampled areas on sorghum, Johnsongrass, and sugarcane, Harris-Shultz et al. (2017) pooled DNA from several specimens collected only on sorghum and Johnsongrass.

Also, even though these studies tried to make comparisons with populations previously described by Nibouche et al. (2014), because they did not use the same molecular markers they could not solve the question of whether the populations causing the actual outbreaks on sorghum is genetically distinct from populations previously present on sugarcane.

To solve this puzzle, Nibouche et al. (2018) used nine microsatellite markers and cytochrome oxidase I (COI) sequencing to compare the genetic diversity of SCA populations collected in the Americas after the 2013 *M. sacchari* outbreak on sorghum (during 2013 - 2017) to older samples collected by Nibouche et al. (2014) before the pest outbreak (during 2007 - 2009). In this new study, the authors collected samples from nine continental states, Caribbean, Central and South America.

The finding of this study showed that the *M. sacchari* outbreak in the Americas and the Caribbean observed since 2013 belong to populations exhibiting low genetic diversity and microsatellite analysis revealed that most of *M. sacchari* populations collected belonged to a

multilocus lineage that had not been found on the previous United States assessment of *M. sacchari* genetic diversity (Nibouche et al. 2018). The newly described multilocus lineage found throughout the sampled locations consists of one dominant clonal lineage, MLL-F, which colonizes *Sorghum* spp. and sugarcane (no host association). The authors also observed, by COI sequencing and microsatellite analyses, that MLL-F specimens are genetically distinct from specimens collected in Louisiana in 2007. MML-F lineage was the only lineage collected from Texas, Oklahoma, Arkansas, Georgia, Alabama, Tennessee, Florida) and California. Louisiana was the only state in which MLL-D was still collected.

Louisiana was the only continental state sampled both in 2007 – 2009 (Nibouche et al. 2014) and in 2013 – 2017 (Nibouche et al. 2018). A major shift, from all genotyped specimens belonging to MLL-D in 2007, to the majority of samples belonging to MLL-F was observed (Nibouche et al. 2018). Moreover, in the 2013-2017 specimens from Louisiana, when COI barcodes were used to characterize *M. sacchari* collected from the continental United States on sorghum, most of the specimens were determined as haplotype H1, whereas in 2007 the sole haplotype observed in Louisiana was H3 (Nibouche et al. 2018).

Finally, the newly described multilocus lineage MLL-F comprised eight multilocus genotypes, with genotype Ms50 representing 90% of the MLL-F specimens. This result (Nibouche et al. 2018) corroborates with Harris-Shultz et al. (2017) results that *M. sacchari* populations in the United States consist of a dominant genotype (i.e., Ms50).

This latest discovery of one predominant genotype infesting sorghum in the United States, indeed suggests an introduction of a non-native genotype. Nibouche et al. (2018) indicate that MLL-F is a new invasive genotype introduced into the Americas either from Africa or Asia.

However, some events can occur on parthenogenetic lineages that introduce changes in a population. Mitotic recombination, in which dissociation or recombination of chromosomes (cross-over), somal clonal mutation, DNA methylation, and post-transcriptional and translational changes might also generate genotypes favoring sorghum over sugarcane exploitation (Dixon 2012, Loxdale and Lushai 2007).

2.6. Management of aphids on sorghum

In sorghum, the primary concern is the losses caused by *M. sacchari* feeding and photosynthate removal. In the United States *M. sacchari* management is focused on scouting and applying insecticides once thresholds are reached.

In an integrated pest management strategy, the first steps are proper identification and scouting of the pest. Differentiation of *M. sacchari* from other aphids attacking sorghum is based on morphology. *Melanaphis sacchari* is the only aphid species attacking sorghum that is tan to light yellow and possess dark cornicles, tarsi, and antennae (Blackman and Eastop 2000, Bowling 2016a). For Louisiana, Bowling et al. (2016b) developed a sampling protocol for early detection of *M. sacchari* in sorghum fields. The detection protocol includes weekly inspection of 50 row ft sampled each side of the field, as well as sites with Johnsongrass. Once *M. sacchari* infestation is detected, scouting is recommended twice weekly to determine if insecticide treatment is needed (Bowling et al. 2016a). In Mississippi, Catchot et al. (2015) recommends starting insecticidal control when 20-30% of the field is infested with *M. sacchari*, while in Arkansas, treatment is recommended when 25% of the plants are infested with more than 50 aphids per leaf (Seiter et

al. 2015). However, sampling time and economic thresholds must consider ecological and biological aspects of the system (Metcalf and Luckman 1994). For instance, above 25°C scouting twice weekly may not be enough to apply treatment measures before the economic threshold is reached (see Chapter 3).

Since 2015, foliar applications of Transform® (Dow AgroSciences, Sulfoxaflor (nicotinic acetylcholine receptor competitive modulators)) and Sivanto® prime (Bayer, Flupyradifurone (nicotinic acetylcholine receptor competitive modulators)) received U.S. Environmental Protection Agency approval for use in most southern states specifically for control of *M. sacchari* on sorghum. (NASS, USDA 2017). Both these insecticides provide up to 98% mortality (Buntin and Roberts 2016, Seiter 2016) and appear to be nontoxic to *Hippodamia convergens* (Colares et al. 2017).

Even though the use of insecticide is the primary control being used in North America, other approaches for *M. sacchari* management have been studied. The use of natural extracts via foliar application on sorghum was studied by Yadav et al. (2016), and compounds such as extracts of Neem (*Azadirachta indica* A. Juss), Jatropha (*Jatropha curcas* L.), Amla (*Emblica officinalis* Gaertn.) and Drumstick (*Moringa olifera* Lam) successfully controlled *M. sacchari*. Field observations reveal an abundant diversity of natural enemies (lady beetles, brown and green lacewings, hoverflies, predatory hemipterans and parasitoids) (Bowling et al. 2016a), and experimental data showed that indigenous predators (*Coleomegilla maculata* DeGeer, *Hippodamia convergens* Guérin-Mèneville, *Chrysoperla carnea* Stephens, and *Orius insidiosus* Say) have great potential to provide sustainable biological control (Colares et al. 2015). The investigation of different strategies must be encouraged. However, so far, the naturally occurring

population of natural enemies has not been able to suppress *M. sacchari* on sorghum, and the use of natural extracts still needs improvement. The use of insecticide spraying alone is not always effective against massive populations. Pest resurgence and insect resistance development can occur when pesticides are the only strategy being used (Metcalf and Luckman 1994).

The use of resistant sorghum lineages has also been investigated (Teetes et al. 1995, Sharma et al. 2013, Sharma et al. 2014, Armstrong et al. 2015, Brewer et al. 2017, Szczepaniec 2018). Sorghum genotypes resistant to greenbug are a good source of resistance and an opportunity to discover materials with resistance to sugarcane aphid. Many different sorghum genotypes are available for research, and their investigation can detect different levels of resistance to *M. sacchari*.

2.7. Host plant resistance

Over the evolution of organisms, mechanisms to regulate populations, communities, and ecosystems evolved concomitantly. In natural systems, insect population control is maintained both by predators and parasitoids (top-down regulation) and by the plant itself (bottom-up regulation). Thus, plants possess traits by which they reduce the impact of herbivory, regulating insect population. Host plant resistance (HPR) is the set of traits by which plants regulate insect populations (Hunter and Prince 1992, Hunter et al. 2001).

The expression of resistance in a plant is a unique result of the complex interactions between the plant and the insect. Thus, HPR can be viewed as the plant traits that alter this insect-plant interaction towards diminishing either injury (physical removal of tissues or nutrients

by the insect), or damage (impact on plant fitness or yield) (Smith and Clement 2012, Stout 2013, Stout 2014).

Painter (1951) defined plant resistance to insects as the heritable traits affecting the level of damage caused by the insect, representing the capacity that certain plants possess to reach greater production in terms of yield than other plants under the same conditions. The classification of Painter (1951), modified by Kogan and Ortman (1978), categorizes plant resistance to insects in non-preference, tolerance, and antibiosis, and such categorization is still useful in some HPR studies. Smith (2005) redefined HPR as the sum of constitutive, genetically inherited qualities that result in a plant of one cultivar or species being less damaged than a susceptible plant lacking these qualities. Later, Stout (2013) redefined resistance in a dichotomous framework in which resistance denotes plant traits that reduce the extent of the injury done to a plant by a herbivore, whereas tolerance encompasses plant traits that reduce the amount of fitness or yield loss per unit injury. However, in all the definitions, the common ground is that resistance is an inherited genetic condition that can be selected and manipulated, producing plants ranging from highly resistant to highly susceptible.

Manipulation of resistance traits in crop plants to control insect pest is an essential branch of the Integrated Pest Management (IPM). However, over time and with the development of insecticides, breeding programs have focused on yield increase in detriment of insect resistance. Yield in detriment of insect resistance resulted in crops highly susceptible to several pests and consequently overuse of pesticides and increasing of insecticide resistance in insects (Smith 2005, van Emden 2007).

Development of plant resistant varieties or cultivars traditionally involves four steps: screening, categorization, breeding, and implementation (Stout 2014). The first step involves the evaluation an array of plant materials, which can be lines, accessions, cultivars, etc., to detect resistance to a determined insect (Stout 2014). Screening for resistance for aphids depends on the type of resistance.

Screening for antixenosis generally involves releasing alatae individuals over different plant materials and accessing their final choice (van Emden 2007). In most of the studies for determination of greenbug non-preference in sorghum, the methodology consisted of laboratory or greenhouse essays in which the preference of aphids for sorghum genotypes was accessed by planting different genotypes in one pot and releasing a certain number of aphids per plant in the center of the pot. Attraction or repellency of the plants genotypes were then measured by the number of aphids present per plant in intervals varying from 4 to 6 hours after insect release (Schuster and Starks 1973, Teetes et al. 1974, Teetes and Johnson 1974, Schweissing and Wilde 1979, Morgan et al. 1980, Starks et al. 1983, Cruz and Vendramim 1995).

However, for aphids host plant selection involves probing, in which aphids stylets penetrate the plant with constant assessment of plant quality through the sensory system (Tjallingii and Esch 1993, Pettersson et al. 2007). The electrical penetration graph (EPG) technique developed by McLean and Kinsey (1964, 1965, 1967) and improved subsequently (Tjallingii 1978, Kawabe and McLean 1980, Kawabe 1985, Tjallingii 1988, Kimsey and McLean 1987, Backus and Bennett 1992) enables the continuous monitoring of aphid probing activity and plant assessment, facilitating an extensive understanding of host plant selection by aphids (Walker and Backus 2000).

Screening for antibiosis involves identifying whether or not the potential resistant plant negatively affects the population increase of an aphid species. The most common and reliable methodology to study antibiosis resistance on aphids is non-choice assays, in which aphids are confined in clip cages or detached leaves individually or in groups (Dixon 2012, Awmack and Leather 2007, van Emden 2007). The method allows one to assess the negative effects of the plant on the aphid biology. In this method, aphid survival, development time, and fecundity can be combined into populations growth estimations such as the intrinsic rate of increase (r_m) (Birch 1948, Awmack and Leather 2007), population doubling time and mean generation time (Birch 1942, Leather and Dixon 1984, Dixon 1990). Sources of antibiosis resistance for aphids, using this method have been identified for important crops pests, such as *Rhopalosiphum padi* (L.), the bird cherry-oat aphid (Descamps and Chopra 2011, Taheri et al. 2010, Karami et al. 2016), *Sipha flava* (Forbes), the yellow sugarcane aphid (Pallipparambil et al. 2014), and *Schizaphis graminum* (Rondani), greenbug (Nuessly et al. 2008, Vakhede and Safavi 2014, Shahrokhi et al. 2010).

Categorization of resistance is more complex on aphid-plant interactions. For instance, a decreased performance due to phloem avoidance (antixenosis) would be seen as antibiosis because the outcome of not feeding is starvation and therefore lowered biological parameters (Pettersson et al. 2007, van Eden et al. 2007). Also, in choice tests, it is known that any repellent compound can cause aphid take off, but this response is switched off after a while (Storer et al. 1996). The EPG technique is the only tool able to categorize plant resistance to aphids on the basis of true antibiosis, which means that aphids are feeding at the same rate on two plants but performance is negatively affected in one of them (Alvarez et al. 2006, Pettersson et al. 2007).

Mechanism of antixenosis resistance for aphids includes, repellent colors (Radcliffe and Chapman 1966, Pettersson et al. 2007), deterrent compounds (Givovich and Niemeyer 1995, Dreyer and Campbell 1987), glossiness or waxiness of the leaves (White and Eigenbrode 2000, Bergman et al. 1991, Ellis et al. 1996), local necrosis (van Emden 1978, Belefantmiller et al. 1994) and non-glandular trichomes (Stoner 1992, Webster et al. 1994, Simmons et al. 2005). Antibiosis resistance for aphids usually comprises glandular trichomes (Ashouri et al. 2001, Simmons et al. 2005), toxic compounds (Leszczynski et al. 1989, Argandona et al. 1983, Fuentes-Contreras and Niemeyer 1998, Hansen 2006), and nutritional factors (Weibull 1994, Ponder et al. 2000, Akbar et al. 2014).

Additionally, the categories of resistance can be subdivided into induced or constitutive, direct or indirect (Stout 2013). Induced resistant traits are traits only expressed (or more strongly expressed) after detection of injury by the plant, while in constitutive resistance the plant constantly expresses traits of resistance. Direct resistant traits affect either the biology or behavior of the insect itself, while indirect resistance traits affect natural enemies (Stout 2013). The next step in the development of plant resistant varieties is breeding. Once plant resistance has been identified breeding incorporates the trait into a genetic background suitable for commercial production (Stout 2014). The last step is the implementation of the resistant plant into integrated pest management program. The decision if the resistance in a cultivar will be the only strategy to control the pest, or if it will be used in integration with any other practice, will depend if the resistance is strong enough to reduce the impact of herbivorous arthropods on crop yield or quality (Stout and Davis 2009, Stout 2013, Stout 2014).

In addition, to the complexity of resistance itself, plants differ in their resistance reaction to the different aphid species (Webster and Ponder 2000, Pitino and Hogenhout 2013, Medina-Ortega and Walker 2013, Rodriguez et al. 2017). For instance, barley shows stronger callose deposition throughout the plant when infested with Russian wheat aphid, *Diuraphis noxia*, than when infested with bird cherry-oat aphid (*R. padi*) (Saheed et al. 2009). The same aphid species can cause different reactions in different plants. Pitino and Hogenhout (2013) found that specific salivary effectors of *M. persicae* produced different performance responses in a plant-specific way. Rodriguez et al. (2017) explained that the ability of *M. persicae* Mp1 protein to reduce a host plant cell trafficking protein is specific to *M. persicae* and its hosts. Therefore, HPR is aphid-plant species specific and cannot be generalized, with each system being independent.

2.7.1. Resistance to aphids in sorghum

On sugarcane, resistance to *M. sacchari* has been studied as a management option. Akbar et al. (2010, 2011) compared resistance to *M. sacchari* in five commercial cultivars and observed that HoCP 91–555 was the less susceptible cultivar. Fartek et al. (2012) demonstrated resistance to *M. sacchari* in one sugarcane cultivar, and then Fartek et al. (2014) screened 181 sugarcane genotypes to *M. sacchari* resistance and confirmed the resistant status of three genotypes.

On sorghum, soon after the first greenbug outbreaks in the late seventies, greenbug-resistant sorghum hybrids were developed. Those hybrids were selected for both tolerance (Hackerott et al. 1969, Schuster and Starks 1973, Morgan et al. 1980, Bowling and Wilde 1996, Cruz and Vendramim 1995) and resistance (Morgan et al. 1980, Cruz and Vendramim 1995, Harvey and Thompson 1988, Cruz and Vendramim 1989, Bowling and Wilde 1996).

Among the various compounds produced by sorghum plant, tannins and dhurrin (a cyanogenic glucoside) are the main portions of plant defense mechanisms. Cyanogenic glucoside production by sorghum seedling has been known for some time (Dustan and Henry 1903), and the cyanogenic glucoside dhurrin has been the target for many studies and reviews (MacFarlane et al 1975, Saunder and Conn 1978, Kojima et al 1978, Nicollier et al 1983, Halkier et al. 1989, Kahn et al 1997, Busk et al. 2002, Kristensen et al. 2005, Gnanasekaran et al. 2016, Muller et al. 2016). Plants producing cyanogenic compounds are bitter, which by nature constitute an insect feeding deterrent (Patton et al. 1997, Gleadow and Woodrow 2002). However, some studies show that glucosides may have no effect on herbivory, or even act as phagostimulants for specialist insects (Gleadow and Woodrow 2002). In sorghum leaves the vacuole is the site of accumulation of the cyanogenic glucosides while the enzyme remains in the cytoplasm (Saunder and Conn 1978). After the contact of the glucoside with the glucosidase enzyme, the sugar fraction of the molecule is released, forming cyanohydrin, an unstable compound that will generate the cyanuric acid (Saunder and Conn 1978). The spatial separation between cyanogenic glucoside (substrate) and glucosidase (enzyme) prevents the formation of the toxic compound inside plant cells, thus upon tissue wounding the substrate and enzyme are brought together and cyanogenesis occurs (Saunder and Conn 1978, Bennett and Wallsgrove 1994, Vetter 2000).

When sorghum plants producing cyanogenic glucoside are damaged by chewing insects the result is a rapid release of cyanic acid, which is thought to deter feeding of various insects (Woodhead and Bernays 1978, Jones 1962, Gleadow and Woodrow 2002). Although artificial diets containing dhurrin act as a deterrent to greenbug feeding (Dreyer et al. 1981), aphid feeding does not disrupt cell content, and the punctures during pathway phase do not cause any damage

to the cell (Tjallingii 1978, Tjallingii 1985, Tjallingii and Esch 1993), thus dhuririn probably does not account for aphid resistance on sorghum plants.

In most plants, during aphid feeding there is an increase in transcription of salicylic acid-dependent pathogenesis-related genes and decrease in the induction of methyl jasmonate (MeJA)-regulated defense genes, which has more resemblance with pathogen infection than with insect infestation. However, on sorghum, *Schizaphis graminum*, greenbug, feeding-induced JA-regulated genes, such as protein inhibitors and lipoxygenase (Zhu-Salman et al. 2004). In infestation tests, Zhu-Salman et al. (2004) showed that the induction of JA-regulated pathways was effective in plant defense against greenbug. Similarly, Losvik et al. (2017) showed that overexpression of lipoxygenase on barley was deleterious for *R. padi*, the bird cherry-oat aphid.

Even though on sorghum the extensive use of certain varieties led to the development of greenbug biotypes, the benefits of HPR as a management tool go beyond avoiding or diminish plant damage. Host plant resistance deployment reduces population levels, while do not interfere with other levels of the ecosystem (natural enemies, for instance), do not promote environment disequilibrium, has a cumulative and persistent effect and does not require sophisticated technologies for implementation (Smith 2005, Stout and Davis 2009, Stout 2014).

2.7.2. Aphid plant resistance characterization

As we discussed before, after reaching a plant, aphid behavior is affected by plant physical and chemical characteristics (Powell et al. 1999, Storer et al. 1996, Powell et al. 2006). For instance, *Aphis fabae* Scopoli (Hemiptera: Aphididae) (a successful polyphagous species), after landing on a plant, has a rapid mechanism for rejection of unsuitable host plants, leaving a plant

as soon as any repelling odor is sensed (Storer et al. 1996). Therefore, characterization of plant resistance for aphid is a complex subject, the proximity of different genotypes may result in susceptible plants being regarded as resistant. On the other hand, a resistant plant may be regarded as susceptible because after a while in a plant the aphid response to repellent odors is switched off (Storer et al. 1996, Powell et al. 2006, Pettersson et al. 2007)

The deterrence of an insect on a plant can occur at several levels and it is not as a choice based only on olfactory and/or visual cues. Current methods such as electroantennogram (EAG) can detect aphid responses to plant surface compounds at the level of antennal olfactory receptors (Hardie et al. 1994, Visser and Piron 1997, Park et al. 2000). However, in piercing-sucking insects, most of the plant recognition and acceptance occurs internally during stylet penetration into plant tissues (Powell et al. 2006, Pettersson et al. 2007).

In aphids, probing comprises stylet insertion and continuous plant penetration. Probing is a process in which the aphid samples intracellular content of plant tissues, and it involves gustatory monitoring and discrimination (Backus 2000). During the probe, samples of intracellular content are transported from the stylets to the pharyngeal gustatory organ, which has about 100 gustatory cells in total (Wensler and Filshie 1969). In this organ, the decision of continuing plant penetration or withdrawn the stylets is made (Pettersson et al. 2007). Many plant factors are involved in host plant acceptance or preference, as aphid-feeding behavior involves an intimate relationship with its host (Powell et al. 1995, Gabrys et al. 1997, Powell et al. 1999, Schwarzkopf et al. 2013). Therefore, for the study of preference in aphids, the most comprehensive technique is the electrical penetration graph (EPG), which elucidates the actual feeding behavior once the aphid penetrates the plant tissue.

Through a Direct-Current system (DC-EPG), plant and insect become part of an electrical circuit. A gold wire is attached to the back of the aphid and glued with silver paint, and a wire is inserted into the soil of a potted plant. The circuit is completed when insect stylets penetrate the plant, and fluctuations in voltage and electrical resistance are recorded as distinct patterns, called waveforms (Walker 2000). The major waveforms distinguished in EPG recordings are intercellular activity, intracellular puncture, and stylet activity, salivation on sieve element, feeding on sieve element and xylem sap ingestion (Tjallingii and Esch 1993, Walker 2000). Also, the known stylet penetration rate of one cell layer per 2 minutes allows EPG studies to locate plant factors affecting aphid behavior (van Hoof 1958, Gabrys et al. 1997). For instance probes shorter than 30 seconds indicates factors of the epidermis causing stylet withdrawal, while probes longer than 30 seconds but shorter than 3 minutes indicates stylet withdrawn from the mesophyll tissue (Gabrys et al. 1997, Caillaud and Via 2000, Schwarzkopf et al. 2013)

Thus, EPG is the primary tool to investigate insect-plant interaction. However, published data on *M. sacchari* feeding behavior are rare. The previous studies on sugarcane revealed that *M. sacchari* rapidly recognizes the host plant and start pathway phase almost immediately, but sugarcane resistant cultivars delayed the first contact to phloem and inhibited sustained feeding (Fartek et al. 2012, Akbar et al. 2014).

Due to the recent infestation of a new *M. sacchari* genotype on sorghum in the United States, effective management programs are needed to reduce the area-wide populations of *M. sacchari*. However, literature is sparse on several aspects of this *M. sacchari* genotype biology and ecology.

Thus, this project sought to elucidate the effect of constant temperatures on *M. sacchari* population dynamics. It also aimed to answer some questions as for how this genotype feeds on sorghum, and if this aphid can accept some of the host plants reported on the literature, and based on what this aphid rejects non-host plants. It was also directed at the evaluation of sorghum plant introduction genotypes for resistance to *M. sacchari* under laboratory, greenhouse as well as field conditions. Identification of aphid resistant germplasms and understanding the bases of aphid-sorghum interactions can assist in future sorghum breeding programs, as well as in IPM strategies.

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CHAPTER 3. INFLUENCE of TEMPERATURE on the BIOLOGY and POPULATION PARAMETERS of *Melanaphis sacchari* (ZEHNTER) (HEMIPTERA: APHIDIDAE).

3.1. Introduction

Among environmental conditions, temperature is a major influence on insects, affecting development, sex ratio, and longevity (Harrison et al. 1985, Bleicher and Parra 1990, Davis et al. 2006, Keena 2006). Temperature has a fundamental importance in insects, altering insect physiology, morphology, and overall insect life history (Sisodia and Singh 2002, Angilletta et al. 2004, Pigliucci 2005, Keena and Moore 2010). Temperature also influences behavioral aspects of insects such as mating, spatial orientation, and walking speed (Langer et al. 2004, Colinet and Hance 2009). Morphological and physiological plasticity due to temperature changes are often observed through alterations in development time, fecundity, and size. At lower temperatures, development is often extended while, at higher temperatures, the adult insect is smaller and has a faster development (Atkinson 1994, Angilletta and Dunham 2003, Angilletta et al. 2004, Pigliucci 2005, Sibly et al. 2007). Alterations in development time and size are frequently related to changes in metabolic rates, as increasing temperatures result in higher enzyme activity, requiring more energy to complete development (Angilletta 2009, Kingsolver and Huey 2008). Effects on oxygen consumption and carbon excretion are another result of temperature; respiration increases in response to increasing temperature (Neven 1998). Besides the metabolic and respiratory alterations, temperature changes also affect the nervous and endocrine systems of insects (Neven 2000). Furthermore, temperature affects the plant host, altering food quality

for herbivores, which may interfere with the insect's response to temperature (Acreman and Dixon 1989).

In hemipterans, aphids are set apart by their distinct life cycle, alternating asexual and sexual reproduction. The aphid life cycle confers the unique ability to assign different roles to different generations, overcoming harsh winter conditions or exploiting highly suitable summer conditions. Temperature plays an important role in aphid life history, influencing development rate, size, fecundity, polymorphism, mating, and migration (Lees 1963, Dixon and Glen 1971, Dixon 1972, Leather and Dixon 1982, Liu 1994, Collins and Leather 2001, Müller et al. 2001, Dixon 2000). When compared to other insect groups, aphids exhibit a much greater phenotypic plasticity due to environmental effects. Therefore, growth and development rates of individual aphids have been reliable tools to predict aphid population dynamics (Leather and Dixon 1984, Acreman and Dixon 1989, Dixon 1990, Dixon 2000).

Aphids cause both direct and indirect injury. The direct injury is due to photosynthate reduction and consumption of plant resources, leading to plant weakening, yellowing, or stunting (Breen and Teetes 1990, Webster 1990, Kindler and Hammon 1996, Dixon 2000). Moreover, aphid feeding can cause deformation of leaves and fruit (Varn and Pfeiffer 1989). Indirect injury results from honeydew production (sugary aphid excretion), toxin injections, and virus transmission (Drees and Jackman 1998, Reynolds 1999).

The sugarcane aphid, *Melanaphis sacchari* (Zehnter), has historically been an important sugarcane (*Saccharum officinarum* L.) pest in the United States, vectoring *Sugarcane yellow leaf virus* (Schenck and Lehrer 2000, Singh et al. 2004). *Melanaphis sacchari* reproduces asexually in the United States and is thought to overwinter as nymphs or adults in remnant sorghum,

Sorghum bicolor (L.) Moench ssp. *bicolor*, Johnsongrass (*Sorghum halepense* L.) and sugarcane (Singh et al. 2004, Bowling et al. 2016). In 2013, outbreaks of *M. sacchari* on sorghum were reported in Texas, Louisiana, Oklahoma, and Mississippi (Villanueva et al. 2014, Bowling et al. 2016). In 2014, *M. sacchari* tripled its range, reaching 12 sorghum producing states with infestation occurring early season. In 2015, all of the 17 states producing sorghum in the United States reported major infestations with *M. sacchari* (Villanueva et al. 2014, Kerns et al. 2015, Bowling et al. 2016). In Louisiana and Texas, aphid population growth can reach over 900 aphids per leaf with yield declines of 60 to 100%, requiring up to four insecticide applications to control *M. sacchari* infestations (Brewer et al. 2017). In the first years of infestation, losses incurred due to *M. sacchari* infestation in sorghum ranged from \$1 million in Alabama to as high as \$35 million in Texas where \$10.5 million was spent on *M. sacchari* control alone. In Louisiana and Mississippi where 100% of the sorghum fields were infested and 75% of the fields required chemical control, \$7.2 and \$3.5 million were spent, respectively, to control this pest (Brewer and Gordy 2016). This uncommon outbreak was confirmed, by genotyping with microsatellite makers and COI barcoding, to be associated with one biotype that has become widespread in the United States (Harris-Shultz et al. 2017, Nibouche et al. 2018).

Estimates of intrinsic rate of increase (r_m) of aphid species can be used to investigate the effects of host quality, plant viruses, sub-lethal insecticides, and temperature (Leszczynski et al. 1989, Fereres et al. 1989, Kerns and Gaylor 1992, Yue and Liu 2000, Davis et al. 2006). *Rhopalosiphum padi* (L.), the birdcherry-oat aphid, can have r_m ranging from 0.309 to 0.381 depending on host species (Taheri et al. 2010, Descamps and Chopra 2011, Karami et al. 2016). *Sipha flava* (Forbes), the yellow sugarcane aphid, exhibits r_m rates of 0.231 on sugarcane and

0.258 on *Miscanthus spp.* (Andersson) (Pallipparambil et al. 2014). On different host plants, *Schizaphis graminum* (Rondani), greenbug, shows intrinsic rates of increase ranging from 0.240 to 0.313 (Nuessly et al. 2008, Shahrokhi et al. 2010, Vakhide and Safavi 2014). Wosula et al. (2013) studied the effect of virus infected host plants on *Myzus persicae* (Sulzer), the green peach aphid, life table parameters. For *Ipomoea batatas* L. cultivar Evangeline, green peach aphid r_m was greater on mixed virus-infected plants (0.251) compared to non-infected plants (0.230), and the contrary was observed on *I. hederacea* Jacques, in which greater r_m was found on non-infected (0.319) compared to SPFMV-infected (0.279) plants. Davis et al. (2006) studied the effects of constant and fluctuating temperatures on the intrinsic rate of increase of *M. persicae*, concluding that greatest r_m occurred at 20°C (0.256) under constant temperatures and at 27°C (0.356) under fluctuating temperatures.

Sugarcane aphid is found in a wide range of climates, varying from places where the temperature in winter can be below 0°C to localities with an average temperature of 30 to 35°C (Singh et al. 2004). It is a multivoltine insect and its life cycle is affected by environmental oscillations. On sorghum, this aphid may develop up to 61 generations per year (Chang et al. 1982) and its life cycle can be completed in as fast as 4.3 days or as long as 37 days (Setokuchi 1973). Under 15°C, *M. sacchari* takes 10.9 days to become an adult and at 20°C, 25°C, and 30°C, it takes 7.3, 5.2, and 3.5 days to complete its life cycle, respectively (Setokuchi 1973). In the most recent study, Michaud et al. (2017) observed that *M. sacchari* on sorghum plants took 5 to 6 days to develop at 23°C. Previous work with *M. sacchari* indicated that temperatures over 20°C decreased longevity and fecundity while increasing mortality (Kawada 1995). However, *M. sacchari* r_m is known to increase with rising temperature, increasing 2.5-times with a 15°C

increment (Abe et al. 2011). Populations of *M. sacchari* build up faster in summer than in winter, when an individual female can produce up to 96 nymphs per female (Chang et al. 1982), resulting in infestations of up to 30,000 aphids per plant (van Rensburg 1973b).

Although it is known that temperature affects *M. sacchari* population growth, specific studies detailing the effect of temperature on a colony collected from sorghum associated with the current outbreak in the United States has not been conducted. In addition, before economic thresholds can be developed, information on population growth under simulated field conditions is needed. Therefore, the objective of this study was to study development of *M. sacchari* population dynamics on sorghum under six constant temperatures in order to estimate upper and lower developmental thresholds and optimum developmental temperature.

3.2. Material and methods

Aphid Colony. The sugarcane aphid colony used in these experiments was founded from a single apterae field collected from sorghum at the Louisiana State Agricultural Center Dean Lee Research Station, Alexandria, LA, in July 2014 by J. A. Davis. This colony, designated LSU-SCA14, was maintained on Pioneer 85G85 (Pioneer Hi-Bred International, Inc., Johnston, IA) planted in 10-cm-dia plastic pots containing sterile potting mix (Sun Gro Horticulture, Elma, MB) and 5 g Osmocote (14-14-14), a slow-release fertilizer (The Scotts Company, Marysville, OH). Plants were grown in a Percival E-36L2 Plant Growth Chambers (Percival Scientific, Perry, IA) held at $25 \pm 0.2^{\circ}\text{C}$, $50 \pm 5\%$ RH and a photoperiod of 14:10 (L:D).

Host Plants. Determination of thermal requirements for the sugarcane aphid was performed on Pioneer 85G85. Plants were planted in plastic pots (11.4 x 15.2 x 15.2 cm) (Model Plastic Nursery Pots Azalea Style, Pöppelmann TEKU®, Claremont, NC) using commercial organic soil for seedlings (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) supplemented with 5 g Osmocote (14-14-14). Pots were maintained in the greenhouse at 22 to 28°C under natural lighting from May to October. When the plants were four to six weeks old, leaves were excised and used in the experiment.

Life table experiments on excised leaves. The study was conducted at six constant temperatures (15, 20, 25, 30, 32 and 35°C) in climate regulated chambers (Model I-41VL, Percival Scientific, Perry, IA) using sorghum excised leaves following procedures by van Schelt (1994) outlined in van Lenteren (1997) and adapted by Sampaio et al. (2001). Temperature inside the chambers were monitored each hour using a miniature data logger (Model HOBO Pendant, Onset Computer Corporation, Bourne, MA)

Leaf sections of approximately 2 x 3 cm were placed in 30 mL Solo (Dart Container Corporation, Mason, MI) cups filled with 15 ml of a 0.1% agarose (wt/vol) (RM301-500G Agar Powder Extra Pure, HiMedia, Einhausen, Germany). Leaf sections were placed on the surface of the agarose with the abaxial surface upward and replaced every 4 days for the lower temperatures of 15 and 20°C, every 3 days at 25°C, and every two days at 30, 32 and 35°C. This method avoided dehydration of the leaves and prevented aphids from escaping from the leaf sections. A single apterous adult was placed on the leaf section using a moist hair paintbrush (21 x 3 x 2 cm) (Model ARTZ-8009, Arteza, USA) and allowed to reproduce for 24 h. The adult aphid

was then removed, leaving only one nymph per leaf section. All single first instars were the cohort for that temperature regimen for the duration of the experiment; 50 nymphs per cohort replicated in three separate experiments. The cohort was evaluated every 24 hours until death. Development time, juvenile mortality, survivorship, fecundity, and longevity were recorded and measured.

Age-specific survival (l_x) and fecundity (m_x) were calculated for each temperature. Net reproductive rate, R_o , defined as the product of age-specific survival and age-specific fecundity, was calculated using the formula $R_o = \sum l_x m_x$, where l_x is the proportion of females alive on a given day, and m_x is the mean number of female births on that day. The intrinsic rate of increase, r_m , ($\sum e^{-r_m} l_x m_x = 1$), finite rate of increase ($\lambda_F = e^{r_m}$), mean generation time [$T_G = \ln R_o / r_m$], and doubling time ($DT = \ln(2) / r_m$) of a generation were estimated according to Birch (1948). Jackknifing procedure was used to estimate r_m standard error. This procedure is based on recombining the original data and calculating pseudo-values of r_m for each recombination of the original data and estimating the mean value and standard error of r_m from the resulting frequency distribution of pseudo-values in accordance with Meyers et al. (1986). Pseudo-values of r_m values were used to estimate values for mean generation time, net reproductive rate, doubling time, and finite rate of increase.

Statistical Analysis. The biological variables (longevity, development time, nymphs per female, reproductive period, nymphs per female per day, and juvenile mortality) were analyzed using a randomized block design. Proc Mixed procedures in SAS 9.4 (SAS, Institute Inc., Cary, NC, 2013) were used for all datasets and Tukey-HSD analysis at 0.05% of significance allowed us to compare

the least square means and determine whether temperature treatments were significant for each variable.

Mean generation time, net reproductive rate, doubling time, and finite rate of increase were analyzed by one-way ANOVA in PROC MIXED with host plants as independent fixed factors. Treatment means were separated by the Tukey-HSD test ($\alpha = 0.05$) (SAS 9.4 Institute Inc., Cary, NC, 2013). Age-specific survival graphs were plotted using SigmaPlot (SigmaPlot 14.0, Systat Software Inc., San Jose, CA, USA, 2018).

Temperature-dependent thresholds under constant temperature regimes were estimated using a Logan model (Logan et al. 1976) as modified by Lactin et al. (1995), as seen below;

$$r(T) = e^{\rho T} - e^{[\rho T_{max} - (T_{max} - T)/\Delta]} + \lambda$$

Where $r(T)$ is the mean developmental rate at temperature T (°C). Fitted parameters ρ (rate of increase at optimal temperature), T_{max} (upper developmental threshold), Δ (difference between optimal and upper developmental threshold), and λ (which allows the curve to intercept x-axis), were estimated using Marquardt's method on PROC NLIN (SAS 9.4, 2013).

3.3. Results

Life Table Analysis. Temperature affected the days to reproductive adult of *M. sacchari* ($F = 510.12$; $P < 0.0001$). *Melanaphis sacchari* reached adulthood faster when it was reared at both 25 and 30°C. Days to reproductive adult of *M. sacchari* decreased as the temperature increased to 25°C (Table 3.1). Development time decreased 4.4 days when the temperature increased from

15°C (12.2 days) to 20 °C (7.8 days) ($F = 19.48$; $df = 5, 149$; $P > 0.0001$) and decreased 3.1 days when the temperature increased again from 20°C (7.8 days) to 25°C (4.8 days) ($F = 28.61$; $P > 0.0001$). Differences were not detected in development time when the temperature increased from 25 to 30°C (4.8 days), however, at 32°C (5.9 days) the development time increased by approximately one day ($F = 10.15$; $P < 0.0001$). The aphid was not able to complete development at a constant 35°C. The mortality of the various nymphal instars was below 20% between 15 and 25°C (Table 3.1). Above 25°C, juvenile mortality increased ($F = 37.92$; $P < 0.0001$) with each temperature increment, reaching 40% and 68% at 30°C and 32°C respectively. At 35°C, all the individuals died before reaching adulthood resulting in 100% juvenile mortality (Table 3.1).

The amount of time in which the female remained reproductively active (reproductive period) was affected by temperature ($F = 152.15$; $P < 0.0001$) (Table 3.1). The longest reproductive period, 18.8 and 15.3 days, occurred at 15 and 20°C, respectively. Raising the temperature to 25°C decreased reproductive activity to 8.9 days ($F = 6.63$; $P < 0.0001$). Females reduced their reproductive period from 8.9 days at 25°C to 1.5 days at 30°C ($F = 12.29$; $P < 0.0001$); however, the difference was not detected in the reproductive period when the temperature increased from 30 to 32°C.

Table 3.1. Biological variables of *Melanaphis sacchari* under different constant temperatures on grain sorghum. Means followed by a different letter are significantly different by Tukey-Kramer test at 5% probability.

Temperature (°C)	n	Immature mortality (%)	Development time (d)	Reproductive period (d)	Nymphs per female	Longevity (d)
15	150	16.6 ± 0.0 a	12.2 ± 0.2 a	18.8 ± 1.2 a	36.4 ± 2.4 b	32.3 ± 1.5 a
20	150	13.3 ± 0.1 a	7.8 ± 0.1 b	15.3 ± 0.9 a	49.8 ± 2.8 a	25.8 ± 1.1 b
25	150	14.0 ± 0.1 a	4.8 ± 0.1 d	8.9 ± 0.6 b	40.0 ± 2.6 b	15.8 ± 0.7 c
30	150	40.0 ± 0.1 b	4.8 ± 0.1 d	1.5 ± 0.4 c	4.0 ± 1.4 c	6.5 ± 0.4 d
32	150	68.6 ± 0.1 c	5.9 ± 0.1 c	1.8 ± 0.3 c	5.1 ± 1.3 c	5.8 ± 0.5 d
35	150	100.0 ± 0.0 d	-	-	-	2.8 ± 0.3 e

Temperature treatments affected fertility of *M. sacchari* females ($F = 172.15$; $P < 0.0001$) (Table 3.1). The greatest production of nymphs per female was at 20°C; females produced an average of 49.8 nymphs, ranging from 4.0 to 111.0 nymphs per female. High fecundity rates also occurred at 15 and 25°C, with 36.4 and 40.0 nymphs/female, respectively. Increasing the temperature to 30°C caused a decrease ($F = 13.94$; $P < 0.0001$) in nymph production per female. At 30°C and 32°C, females had the lowest nymph production of 4.1 nymphs and 5.1 nymphs

produced per female, and at these temperatures, many females reached adulthood but did not produce any nymphs.

The average lifespan from day one until death (longevity) was affected by temperature and decreased with increasing temperature ($F = 250.02$; $P < 0.0001$) (Table 3.1). The longest *M. sacchari* longevity was achieved at 15°C with insects living for 32.3 days on average and the shortest longevity was observed at 35°C with insects being alive for an average of 2.8 days.

Age-specific survivorship (l_x) decreased linearly with the increase in temperature (Fig. 1). At 15°C, the greatest age-specific survivorship was observed, 75 days. Observations up to 53 days at 20°C and up to 31 days at 25°C were recorded. Even at the highest constant temperatures of 30 and 32°C, aphids could survive for approximately 20 days, but at 35°C, only a few individuals survived until day 10. Survival started to diminish after the seventh day at a temperature of 20°C, at the fourth day at 25°C, and at the second day at the other temperatures (Fig. 3.1).

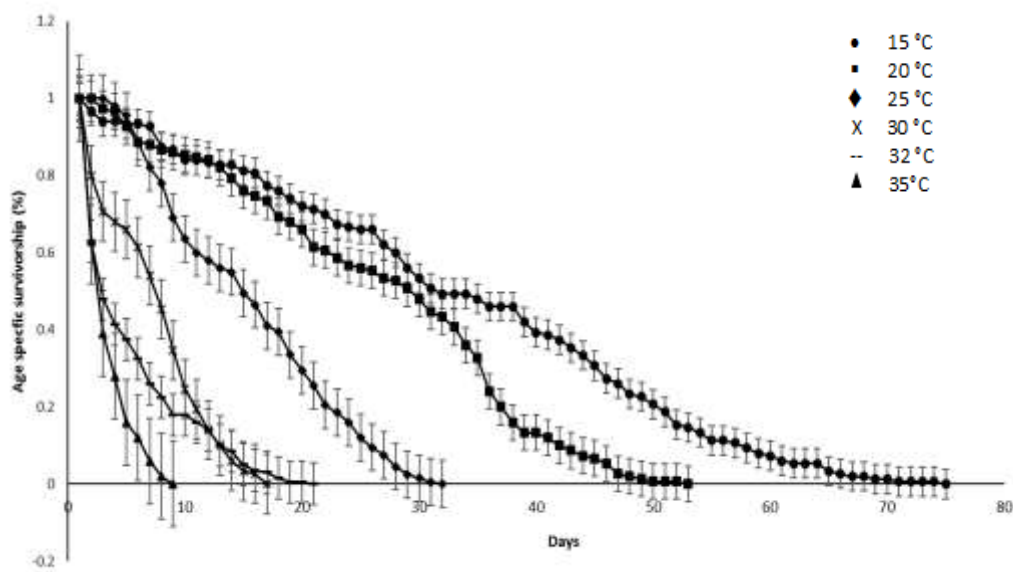


Figure 3.1. Age-specific survivorship of *M. sacchari* under different constant temperatures on grain sorghum

The highest net reproduction rate of 50.4 occurred at 20°C (Table 2). At 15 and 25°C, the R_0 values were 31.1 and 34.0, respectively, and the lowest R_0 values of 3.6 and 4.5 were found at 30 and 32°C respectively. The intrinsic rate of increase was highest at 25°C (0.405 ± 0.030), indicating that population increases fastest at this temperature, while aphids kept at the colder temperatures had lower r_m (Table 3.2). The finite rate of increase (λ_F) was highest at 25°C at 1.5 nymphs/female/day. The maximum and minimum population doubling times (DT) were 5.6 at 30°C and 1.7 days at 25°C (Table 3.2).

Table 3.2. Population parameters of *M. sacchari* under constant temperature regimen on grain sorghum

Temp. (°C)	Intrinsic rate of increase			Net reproductive rate	Mean generation time	Doubling Time	Finite rate of increase
	n	r_m	SE				
15	150	0.197	± 0.006	31.1 ± 0.014 d	17.4 ± 0.027 e	3.5 ± 0.0007 c	1.2 ± 0.0005 b
20	150	0.274	± 0.013	50.4 ± 0.047 e	14.3 ± 0.0017 d	2.5 ± 0.0008 a	1.3 ± 0.0001 c
25	150	0.405	± 0.030	34.0 ± 0.039 c	8.7 ± 0.0021 b	1.7 ± 0.0009 a	1.5 ± 0.0003 d
30	150	0.124	± 0.017	3.6 ± 0.043 a	10.3 ± 0.0021 c	5.6 ± 0.0054 d	1.1 ± 0.0001 a
32	150	0.197	± 0.017	4.5 ± 0.035 b	7.6 ± 0.0015 a	3.5 ± 0.0021 c	1.2 ± 0.00014 b
<i>F</i>				526.452	332.538	294.385	710.268
<i>P</i>				> 0.0001	> 0.0001	> 0.0001	> 0.0001

Thermal requirements. When we fitted the observed data in the Logan Lactin-modified model (1995), the lower developmental threshold for *M. sacchari* development was 8.6°C and the

maximum developmental threshold was 37.8°C, with an optimum development rate at 28.3°C (Table 3.3, Fig. 3.2).

Table 3.3. Estimated parameters of Lactin model for constant temperature regimes

Model	ρ	SE \pm	Tmax	SE \pm	Δ	SE \pm	λ	SE \pm
Constant	0.01081	0.00035	37.808	0.517	2.506	± 0.242	-1.097	± 0.007

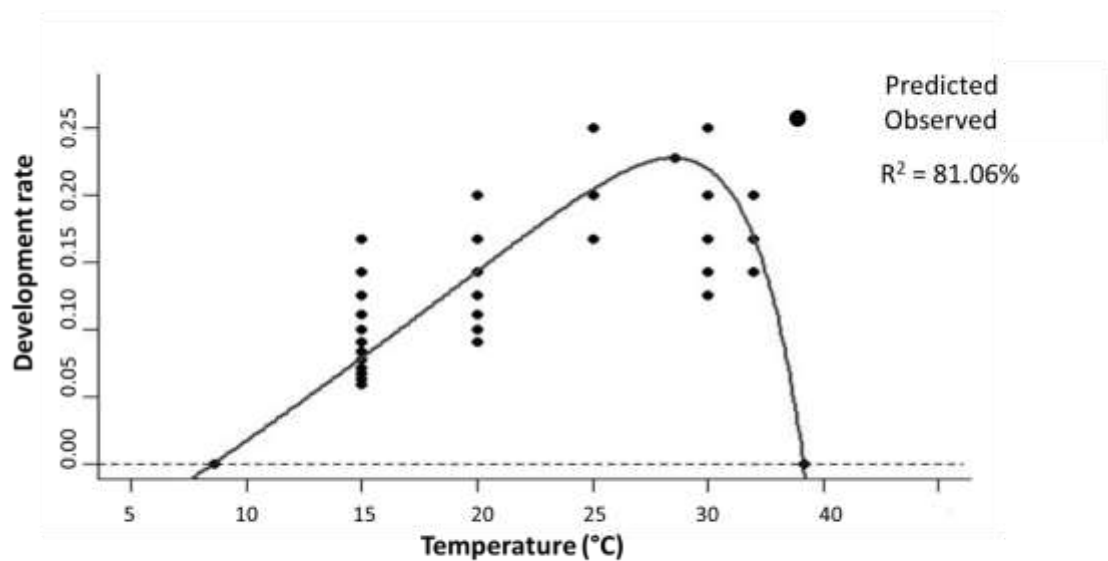


Figure 3.2. Constant temperature dependent development rate Lactin model.

3.4. Discussion

The lower developmental threshold of 8.6°C and the upper developmental threshold of 37.8°C observed in the present study are higher than the threshold for most aphid species. Even though lower developmental thresholds of different aphid species can vary from 3.6°C (Carter et al. 1982, Zhou et al. 1989) to 11.8°C (Bayhan et al. 2005), lower developmental thresholds above 7.0°C are not common in the literature. High lower development thresholds similar to our results for *M. sacchari* are unusual among aphids that colonize sorghum. *Ropalosiphum padi* L. exhibits above average thermal thresholds (Asin and Pons 2001, Ma and Ma 2007), however its lower development threshold does not reach 7.0°C (Elliot and Kieckhefer 1989, Auad et al. 2009, Park et al. 2017) with an optimum temperature for development between 25°C and 28°C (Dean 1974, Asin and Pons 2001) and upper development threshold <30°C (Dean 1974, Elliot and Kieckhefer 1989, Asin and Pons 2001, Auad et al. 2009).

Oliveira et al. (2009a) studied yellow sugarcane aphid under seven constant temperatures ranging from 12 to 32°C and observed a lower thermal threshold of 2.1°C with an optimum temperature for development between 20°C and 24°C. Under seven constant temperatures, ranging from 10 to 33°C, Tofangsazi et al. (2010) observed an optimum temperature for *S. graminum* development and growth of 26°C, and the lower thermal threshold was only 5.7°C. We could not find, in the literature, developmental thresholds for *M. sacchari* on any of its host plants in order to make related comparisons. Our developmental threshold data indicate a great tolerance of *M. sacchari* to elevated temperatures. However, the metabolic and morphological features that allow this species to have optimum development above at 28.3°C are not clear.

Heat shock proteins may be an important component of this condition. Heat shock proteins act as chaperones and prevent protein denaturation under heat stress (Okada et al. 2014, King and MacRae 2015), and species-specific production of heat shock proteins have been reported (Sharma et al. 2007, Wang et al. 2013, Li et al. 2017). On *R. padi*, heat shock proteins were induced when the aphid was exposed to temperatures ranging from 36 to 38°C, indicating that for this species, heat shock proteins are an important component in heat tolerance (Li et al. 2017).

The nervous system is also important in thermal tolerance, involved in perceiving temperature changes and in regulating the responses, adjusting insect behavior and physiology (Clarke 1967). Thus, a greater sensitivity to heat and a rapid response to heat may be some other factors contributing to the high thermal threshold observed in *M. sacchari*. While most aphid species start to suffer in temperatures above 25°C, *M. sacchari* had faster development at 25 and 30°C and higher upper developmental thresholds.

Symbionts, such as *Serratia symbiotica*, are thought to increase aphid heat tolerance (Montllor et al. 2002, Russell and Moran 2006, Heyworth and Ferrari 2015) through nutritional compensation when heat impairs *Buchnera aphidicola*, (Koga et al. 2003, Russell and Moran 2006). Secondary endosymbionts conferring heat tolerance are more frequent in aphids found in tropical areas (Henry et al., 2013). Currently, there is no report of the endosymbionts harbored by *M. sacchari*.

Setokuchi (1973) reported that under 15°C, *M. sacchari* remained 10.9 days in the juvenile stage, and at 20°C, 25°C and 30°C, the aphid spent 7.3, 5.2, and 3.5 days to complete its life cycle, respectively. This former study (Setokuchi 1973) was performed under field conditions and

without statistical analysis, thus differing from our experimental design. For *S. graminun*, Pendleton et al. (2009) and Tofangsazi et al. (2010) found that increasing temperature also decreased development time, with aphids taking up to 32 days to develop at 10°C and less than 5 days under mean temperatures of 28°C. Our low juvenile mortality rates below 25°C corroborates with the recent study by Michaud et al. (2017) that found that on sorghum under $23 \pm 1.5^\circ\text{C}$, *M. sacchari* highest juvenile mortality was 35%. Moreover, the great juvenile mortality at the high temperatures and the inability to complete development at 35°C is a very common observation among aphid species (Dean 1974, Elliot and Kieckhefer 1989, Asin and Pons 2001, Auad et al. 2009) including the sorghum aphids *M. sacchari* (Setokuchi 1973, Kawada 1995) and *S. graminum* (Tofangsazi et al. 2010) on sorghum.

According to Bale (1999), temperature is the main factor affecting the rate-based process (processes affected by the standard metabolic rate) of aphids, and the constant exposure to high temperatures leads to both faster development and greater mortality. Mortality is a result of either the indirect effects of high temperature in the host plant or the direct effect on the insect. The direct effects on the insect are due to the denaturing of proteins or metabolic abnormalities from the reduction of respiration and accumulation of toxic products, and these harmful effects mainly occur if the temperature is held constant (Campbell et al. 1974).

The high fecundity rates we observed between 15 and 25°C agree with observations by Kawada (1995). In addition, van Rensburg (1973b) reported *M. sacchari* fertility rates on sorghum of 85.6 and 96.2 nymphs per female at 22.2°C and 30.5°C respectively, and Chang et al. (1982) observed higher fertility rates during summer than on winter, when females produced up to 96 nymphs. We observed that *M. sacchari* produced the greatest number of nymphs per female at

20°C. Similarly, this was also observed by Abe et al. (2011) on sorghum and on *S. flava* by Oliveira et al. (2009b). However, while in the present study the highest production of nymphs per female at 20°C was 49.8, Abe et al. (2011) observed an average production of 80 nymphs per female at this temperature. In contrast, *M. sacchari* fertility of 25.6 nymphs per female observed on sorghum at 24°C by Lopes-da-Silva et al. (2014) was much lower than the fertility observed at 25°C in the present study, indicating that the same aphid species, on the same host plant, can have different reproductive potentials. These variations may be attributed to biological differences in regionally-adapted clones, plant quality, or other abiotic factors (Leather and Dixon 1982, Acreman and Dixon 1989, Tang et al. 1999). Thus, the unique fertility rates found in this study are probably due to the aphid genetic constitution, which has not been studied before. For instance Pendleton et al (2009) observed that under temperatures of 28°C, *S. graminum* biotype I produced fewer nymphs than biotype E on sorghum plants.

Aphid fertility rates are reported to reach a maximum between 20 and 25°C (Harrington et al. 1995) but above 25°C, aphids produce fewer embryos and have a poor ability to maintain embryo maturation (Leather and Dixon 1982, Carroll and Hoyt 1986, Collins and Leather 2001). This decrease in fertility occurs because aphid somatic growth and reproductive development occurs simultaneously and are greatly affected by environmental conditions. Thus, aphids can respond to actual habitat conditions by altering the reproductive output, either resorbing or ovulating more embryos (Dixon 2000). According to Dixon (2000), aphids that do not expect an improvement in environmental conditions, such as temperature, mature the largest embryos and resorb the smallest ones in an attempt to ensure offspring success. In the present study, the metabolic damages, the increase in energy expenditure, and the increased respiration rates

caused by higher temperatures, undoubtedly, demand the female to use its potential fertility to stay alive and maintain a low immediate fertility rate.

A decrease in longevity with increasing temperature is a common observation among aphids and was expected for *M. sacchari*. Lopez-da-Silva et al. (2014) observed that *M. sacchari* longevity on sorghum varied between 8 and 42 days under 24°C. Likewise, Kawada (1995) reported a decrease in *M. sacchari* lifespan on sorghum above 25°C. In contrast, van Resburg (1973a) did not find differences in *M. sacchari* longevity at a minimum of 22.2°C and a maximum of 30.5°C on sorghum plants. Likewise, for the other aphid species attacking sorghum, *S. flava*, *S. graminum* and *R. padi*, Auad et al. (2009), Oliveira et al. (2009) and Tofangsazi et al. (2010) found that longevity decreases with temperature increase. Beyond the optimum temperature for aphid performance, longevity is rapidly reduced (Dean 1974, Asín and Pons 2001), and this reduction in longevity is related to insect metabolic rates. Increasing the temperature also increases energy demand, thus requiring more energy to complete the insect life cycle what affects lifespan (Kingsolver and Huey 2008, Miller et al. 2009, Angilletta 2009). Colinet et al. (2007) found a negative correlation between temperature and dry mass and between temperature and fatty tissue, which has consequences in fecundity and longevity (Ellers et al. 1998, Casas et al. 2005). Moreover, reductions in overall fitness due to temperature increase may be associated with damages that heat stress causes to the primary aphid endosymbiont, *Buchnera aphidicola*.

Our age-specific survival data agree with those reported in the literature for other aphid species, which observe a linear increase of survivorship with a decrease of temperature (Nowierski et al. 1983, Wang et al. 1997, Morgan et al. 2001, McCornack et al. 2004, Davis et al. 2006, Hough et al. 2017), including *M. sacchari* that reached 50% survivorship at the 18th day at

24°C (Lopes-da-Silva et al. 2004). Moreover, our age-specific results are very similar to that found by Abe et al. (2011).

Intrinsic rate of increase observed in *M. sacchari* on sorghum corroborates previous reports of a greater *M. sacchari* population increment during hotter periods (van Resburg 1973a,b, Chang et al. 1982, Kawada 1995). Lopes-da-Silva et al. (2004) found a rate of increase of *M. sacchari* on sorghum at 24°C of 0.30, a much smaller value than the present study found at 25°C (0.405). We found that r_m doubled when the temperature increased from 15 to 25°C, and the same rate was observed for *M. sacchari* on sorghum by Abe et al. (2011). However Abe et al. (2011) observed that the r_m increased from 0.390 at 25°C to 0.450 under 30°C, while in the present study the r_m decreased from 0.405 to 0.124 when the temperature increased from 25 to 30°C. At 24°C, *M. sacchari* R_0 was 27.70 (Lopes-da-Silva et al. 2004), while in the present study, *M. sacchari* population growth from one generation to the next was nearly 6-times higher at 25°C.

Our results are comparable to observations made by Tofangsazi et al. (2010), in which *S. graminun* had a net reproductive rate of 37.3 at 15°C and 57.89 at 26°C. In contrast, the values are much greater than those observed in *S. flava* by Oliveira et al. (2009b) (10.92 at 16°C and 9.71 at 24°C) and *R. padi* (Auad et al. 2009) (9.92 at 16°C and 13.42 at 24°C). Reports for other cereal aphid species reveals that increasing the temperature in the same range of the present study (15 to 25°C) results doubling of intrinsic rate of increase (Asin and Pons 2001) or increasing r_m as much as 4-times (Auad et al. 2009). Development, survivorship, growth, and fecundity of individual aphids can predict population trends (Leather and Dixon 1984, Acreman and Dixon

1989, Dixon 2000) and this has been used to predict population growth in diverse environmental conditions (Zuniga et al. 1985, Sumner et al. 1986, Warrington et al. 1987, Fereres et al. 1989).

The finding of this study reveals key aspects of this specific *M. sacchari* genotype collected on sorghum under different temperatures that have not been investigated before, and may shed some light on the recent outbreaks in the United States. Although the laboratory conditions of low densities and constant temperature are not consistent with field conditions, the laboratory study can provide valuable information about life history and population dynamics as comparisons of r_m are the most reliable way to predict population performance under different conditions. In addition, in order to estimate economic thresholds, the observations made in the present study are essential. Therefore, these findings will assist in planning control measures. For instance, using the monthly average for the past 15 years (NOAA 2017), *M. sacchari* lower developmental threshold and population parameters found in this study, we predict that in Florida, Georgia, Louisiana, Mississippi and Texas, *M. sacchari* population will start to develop as soon as February and will start to peak in May, requiring earliest scouting, monitoring, and control.

3.5. References cited

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CHAPTER 4. LIFE TABLE and FEEDING BEHAVIOR of *Melanaphis sacchari* (ZEHNTNER) (HEMIPTERA: APHIDIDAE) on DIFFERENT HOST PLANTS

4.1. Introduction

The sugarcane aphid (*Melanaphis sacchari* Zehntner) (Hemiptera: Aphididae) is a cosmopolitan species known worldwide for its losses on sugarcane, *Saccharum officinarum* L. (Blackman and Eastop 2000) and sorghum, *Sorghum bicolor* (L.) Moench (Singh et al. 2004). It is a well-recognized sorghum pest in South America, China, Taiwan, Japan, India, South Africa, and Botswana (Singh et al. 2004). Although *M. sacchari* is often associated only with sugarcane and sorghum, Singh et al. (2004) mention *M. sacchari* feeding on *Cynodon dactylon* L. (bermudagrass), *Miscanthus sinensis* Andersson (ornamental grass), *Oryza sativa* L. (rice), *Panicum colonum* L. (barnyard grass), *Panicum maximum* Jacq. (Hamil grass), *Paspalum sanguinale* Lamarck (hairy crabgrass), *Pennisetum* sp., *Setaria italica* (L.) Beauv. (Foxtail millet), *Sorghum halepense* (L.) Pers. (Johnsongrass), *Sorghum verticilliflorum* (Steud.) Stapf. (Wild Sudangrass), *Zea mays* L. (maize). Additional hosts include the genera *Arthraxon*, *Oryzopsis*, *Themeda*, and *Echinochloa* in the Poaceae family and *Arum* and *Caladium* (Araceae) (Denmark 1988, Blackman and Eastop 2000). Under no-choice laboratory conditions, *M. sacchari* successfully transmitted sugarcane yellow leaf virus, a phloem restricted virus, to wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.), indicating it does feed on these hosts (Schenck and Lehrer 2000, ElSayed 2013). In the United States, *M. sacchari* seems to be restricted to sugarcane, Johnsongrass, and sorghum (Armstrong et al. 2015, Bowling et al. 2016). Although *M. sacchari* infestations in the United States were initially limited to sugarcane in Texas,

Louisiana, Florida, and Hawaii, in 2013, the pest was reported infesting sorghum in Texas, and since then, has become one of the most critical sorghum insect pests.

The spread of *M. sacchari* was rapid, with initial reports in Texas, Louisiana, Oklahoma, and Mississippi in late 2013 (Villanueva et al. 2014, Bowling et al. 2016). In 2014, *M. sacchari* tripled its range, and in 2015, all of the 17 states producing sorghum in the United States reported significant infestations of *M. sacchari* (Villanueva et al. 2014, Kerns et al. 2015, and Bowling et al. 2016). *Melanaphis sacchari* damages sorghum directly by removing photoassimilates and by reducing photosynthetic efficiency due to sooty mold (Singh et al. 2004). In the first year of infestation, the total impact of the *M. sacchari* was estimated at \$7.6 million in Louisiana and \$35 million in Texas (Bowling et al. 2016, Brewer and Gordy 2016, Brewer et al. 2017).

The rapid spread of this aphid on sorghum indicated the possible introduction of a sorghum-specialized biotype into the United States. Harris-Shultz et al. (2017) demonstrated by genotyping with 52 microsatellite makers that this outbreak on sorghum is associated with a predominant biotype that became widespread. The biotype was found at 17 locations in seven states and Puerto Rico (Harris-Shultz et al. 2017). Nibouche et al. (2018) used nine microsatellite markers and cytochrome oxidase I (COI) sequencing to compare the genetic diversity of *M. sacchari* populations collected in the Americas after the 2013 *M. sacchari* outbreak on sorghum (during 2013 - 2017) to older samples collected by Nibouche et al. (2014) before the pest outbreak (during 2007 - 2009). In this study (Nibouche et al. 2018), the authors collected samples from nine continental states in the United States. The findings of this study revealed that most *M. sacchari* populations collected belonged to a multi-locus lineage (MLL-F) that had not been found in the previous global assessment by Nibouche et al. (2014) of *M. sacchari* genetic diversity.

The authors also observed, by COI sequencing and microsatellite analyses, that MLL-F specimens were genetically distinct from specimens collected in Louisiana in 2007. Additionally, the state of Louisiana was the only one in which two multi-locus lineages (MLL-F and MLL-D) were collected. Finally, the newly described multi-locus lineage MLL-F comprised eight multi-locus genotypes (genotypes differing from each other by stepwise mutations), with genotype Ms50 representing 90% of the MLL-F specimens. These results corroborate Harris-Shultz et al. (2017) results that *M. sacchari* populations in the United States consist of a dominant genotype. This *M. sacchari* clone shows extensive distribution and high frequency, and thus, and it is expected to show differential fitness and behavioral responses across distinct host plants and temperatures ranges (Vorburger et al. 2003, Chen et al. 2013, Harrison and Mondor 2011).

In the United States, *M. sacchari* reproduces parthenogenetically and is thought to overwinter as nymphs or adults in remnant sorghum, Johnsongrass and sugarcane (Singh et al. 2004, Bowling et al. 2016, Harris-Shultz et al. 2017). Once the temperature starts to rise, the winter populations start to grow, and *M. sacchari* disperses to summer hosts. In the United States, the sorghum belt runs from South Dakota to Southern Texas. The 5.9 million ha of sorghum in the sorghum belt (Arkansas, Colorado, Kansas, Louisiana, Nebraska Oklahoma, South Dakota, and Texas) grows alongside 43.1 million ha of wheat and maize, 466,100 ha of sugarcane and 14,000 t ha of sweetpotato (*Ipomoea batatas* L.) (USDA, NASS 2018).

In Louisiana, wheat, rice, maize, and sweetpotato may play a role in *M. sacchari* survival and population growth pre-and post-sorghum planting and harvest. Wheat varieties are planted from mid-October to mid-November in northern Louisiana and November in central and southern Louisiana (Lofton et al. 2015). Maize is planted in February or March (Arledge and

Sharpe 2015) and most rice emerges in mid-March (Saichuk 2009). Sorghum is not planted until April through May (Fromme et al. 2017). Thus, there is the potential for these crops to be important bridging species (plants that provide resources over resource-limited times) for *M. sacchari*.

The only study concerning the host range of the *M. sacchari* biotype found in the United States concluded that *M. sacchari* could survive only on grain sorghum and on Johnsongrass (Armstrong et al. 2015). Understanding how *M. sacchari* interacts with other crops within a growing season is needed to achieve a more comprehensive assessment of this new challenge on sorghum production.

The objective of this study was to understand the impact of potential hosts of *M. sacchari* found in the Louisiana agroecoscape. Specifically, this study determined the population dynamics of *M. sacchari* on sugarcane, sorghum, rice, sweetpotato, maize, Johnsongrass, and wheat using life table studies. Additionally, this study elucidated potential mechanisms of host acceptance and use by determining the feeding behavior of *M. sacchari* on these host plants using the electrical penetration graph (EPG) technique.

4.2. Material and methods

Aphid Colony. The *M. sacchari* colony used in these experiments was founded from a single apterae field collected from sorghum at the Louisiana State Agricultural Center Dean Lee Research Station, Alexandria, LA, in July 2014 by J. A. Davis. This colony, designated LSU-SCA14, was maintained on Pioneer 85G85 (Pioneer Hi-Bred International, Inc., Johnston, IA) planted in

10-cm-dia plastic pots containing sterile potting mix (Sun Gro Horticulture, Elma, MB) and 5 g Osmocote (14-14-14), a slow-release fertilizer (The Scotts Company, Marysville, OH). Plants were grown in a Percival E-36L2 Plant Growth Chambers (Percival Scientific, Perry, IA) held at $25 \pm 0.2^{\circ}\text{C}$, $50 \pm 5\%$ RH and a photoperiod of 14:10 (L:D).

Test Plants. Sorghum var. Pioneer 85G85, Johnsongrass var. VNS (Turner Seed, Breckenridge, TX) wheat var. LA841 (Louisiana State University Agricultural Center Wheat Breeding Program), rice var. Cocodrie (Louisiana State University Agricultural Center Rice Breeding Program), sugarcane var. LSU 01-299 (Louisiana State University Agricultural Center Sugarcane Breeding Program), maize var. Sweet G90 (Ferry-Morse Seed Company, Norton, MA) and sweetpotato var. Beauregard (Louisiana State University Agricultural Center Sweetpotato Breeding Program) were used in these experiments. Except for sugarcane and sweetpotato, seeds of each plant species were planted in plastic pots (11.4 x 15.2 x 15.2 cm) (Model Plastic Nursery Pots Azalea Style, Pöppelmann TEKU®, Claremont, NC) using commercial organic soil for seedlings (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) supplemented with 5 g Osmocote (14N-14P-14K). Pots were maintained in the greenhouse at 22 to 28°C under natural lighting. When plants were four to six weeks old, leaves were excised and used in the experiment. Sugarcane seedlings were collected from the Louisiana State University Agricultural Center Sugar Research Station in St Gabriel, LA and Sweetpotato plantlets derived from meristematic tissue culture were planted in plastic pots (11.4 x 15.2 x 15.2 cm) (Model Plastic Nursery Pots Azalea Style, Pöppelmann TEKU®, Claremont, NC) using commercial organic soil for seedlings (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) supplemented 5 g Osmocote (14-14-14) and maintained in the greenhouse

Life Table Studies. The experiments compared aphid development and life traits on the host plants using excised leaves. Studies were conducted at a constant temperature of 25°C in climate regulated chambers (Model I-41VL, Percival Scientific, Perry, IA) using excised leaves following procedures by van Schelt (1994) outlined in van Lenteren (1997) and adapted by Sampaio et al. (2001). The modified procedure comprises: leaf sections of approximately 2 x 3 cm, collected from the middle section of the potted plants, were placed in 30 mL Solo (Dart Container Corporation, Mason, MI) cups filled with 15 ml of a 0.1% agarose (wt/vol) (RM301-500G Agar Powder Extra Pure, HiMedia, Einhausen, Germany). Leaf sections were placed on the surface of the agarose with the abaxial surface upward and replaced every four days. This method avoided dehydration of the leaves and prevented aphids from escaping from the leaf sections. A single apterous adult was placed on the leaf section using a moist paintbrush (21 x 3 x 2 cm) (Model ARTZ-8009, Arteza, USA) and allowed to reproduce for 24 h. The adult aphid and all nymphs but one were then removed, leaving only one nymph per leaf section. All single first instars were the cohort for that aphid-host plant combination for the duration of the experiment; 50 nymphs (each coming from a different female) per cohort replicated three times. The cohort was evaluated daily for deaths, change of instars, and number of nymphs until death of all the individuals.

Life table parameters were calculated for each test plant species following the methods of Birch (1948). Cohorts were checked daily. Age (x), age-specific survival (l_x), development time, and number of progeny per female per day (m_x) were recorded, and age-specific fecundity ($l_x m_x$) was calculated. Life table parameters were calculated as follows:

Intrinsic rate of increase (r_m) was calculated as

$$\sum e^{-r_m} l_x m_x = 1$$

Net reproductive rate (R_o) was calculated as

$$R_o = \sum l_x m_x$$

Finite rate of increase (λ) was calculated as

$$\lambda = e^{r_m}$$

Doubling time (DT) was calculated as

$$DT = \ln(2)/r_m$$

Mean generation time (T_g) was calculated as

$$T_g = \ln R_o / r_m$$

Electrical Penetration Graph. To measure *M. sacchari* probing behavior on each plant species, EPG experiments were conducted in a Faraday cage by using a Giga 4 DC EPG amplifier with 1-giga ohm input resistance and an AD conversion rate of 100 Hz (Wageningen Agricultural University, Wageningen, The Netherlands). A DAS- 800 Digital Acquisition Card (Keithley Instruments, Inc., Cleveland, OH) converted analog signals into digital, which were visualized and recorded using WinDaq/Lite software (DATAQ Instruments, Inc., Akron, OH). Feeding behavior waveforms identifying specific aphid probing activities were identified using the characteristics listed in Tjallingii and Esch (1993). Apterous adults were removed from the laboratory colony and used in feeding behavior studies. A 2-cm length of 18- μ m gold wire (GoodFellow Metal Ltd.,

Cambridge, United Kingdom) was attached to *M. sacchari* dorsum with silver conductive paint (Pelco Colloidal Silver no. 16034, Ted Pella, Inc., Redding, CA). Two test plants (all the same species) were placed randomly within the Faraday cage. Next, two aphids per test plant were then placed on the adaxial side of separate individual leaves (aphids moved freely and were not restricted on upper leaf surfaces), and feeding behavior was recorded for 4 h. This was repeated 10 times; 40 aphids per host species, 120 h of aphid feeding on each host.

The beginning and the end of each EPG waveform (Table 4.1) in all EPG recordings were marked manually and subsequently, the 32 EPG parameters representing aphid probing and feeding behaviors were calculated (Table 4.1). Measured parameters comprised the start and end of each probe, time to start the first probe (Table 1, #5), time to reach phloem phase and to feed (from start of the experiment) (Table 1, #19 and #20), start and end of each individual phloem phase and xylem phase, total numbers of potential drops during the time of the recording (Table 1, #10), number of events for each phase (i.e., number of E1, E2, E2>10min, xylem) (Table 1, #24, 25, 26 and 31).

From the measured parameters we derived global parameters, phloem and xylem phase parameters. Global parameters included proportion of individuals starting penetration (Table 1, # 1), number of probes (Table 1, # 2), the total penetration time (sum of all probing time within a 4-h period) (Table 1, # 3) and non-penetration time (Table 1, #4), number of probes shorter than 30s (Table 1, #8), number of probes shorter than 3min (Table 1, #9), cell puncture frequency per minute (total pds/time probing) (Table 1, #11), cell punctures per probes (total pds/ total number of probes) (Table 1, #12). Parameters before phloem phase of total duration of pathway phase (time probing excluded phloem and xylem phases) (Table 1, #15), numbers of cell

punctures (potential drops) to reach sieve element phase (Table 1, #14), number of probes before first phloem contact (Table 1, #13), time from first probe to first sieve element salivation (Table 1, #21), time from first probe to first phloem feeding (Table 1, #22), time from first salivation to first sustained feeding (Table 1, #23), were also calculated.

Phloem and xylem phase calculated parameters included proportion of individuals showing phloem phase (individual showing any activity/individuals reaching phloem) (Table 1, #16), proportion of individuals showing repetitive punctures on sieve elements (Table 1, #17), proportion of individuals showing sustained phloem feeding (feeding activity longer than 10 min) (Table 1, #18), sum of time spent in each individual phloem phase (E1, E2) (Table 1, #27 and 28), proportion of individuals showing xylem phase (Table 1, #30 and total duration of xylem phase (sum of all time in xylem phase) (Table 1, #32). All parameter calculations were based on studies of Tjallingii and Esch (1993), Gabrys et al. (1997), Caillaud and Via (2000), Tjallingii and Gabrys (1999) and Tjallingii (2006)

Statistical Analysis. The Skewness and Kurtosis values were obtained for the biological data (development time, m_x , average progeny per female, reproductive period, period in each instar, longevity) in PROC MEANS using SAS software version 9.4 for Windows (SAS Institute Inc., Cary, NC, 2013) prior to ANOVA. The highly skewed data (values < -1 or > 1) or the ones with high Kurtosis values (-3.0 to 3.0) were rank transformed (Michel 2014) in PROC RANK (SAS 9.4, Institute Inc., Cary, NC, 2013). Aphid development time, longevity, m_x , average progeny per female, reproductive period, period in each instar and longevity were analyzed by one-way

ANOVA in PROC GLIMMIX with host plants as independent fixed factors. Treatment means were separated by the Tukey-HSD test ($\alpha = 0.05$) (SAS 9.4, Institute Inc., Cary, NC, 2013).

Jackknifing procedure was used to estimate r_m standard errors. This procedure is based on recombining the original data and calculating pseudo-values of r_m for each recombination of the original data, and estimating the mean value and standard error of r_m from the resulting frequency distribution of pseudo-values (Meyers et al. 1986). Pseudo-values of r_m values were used to estimate values for mean generation time, net reproductive rate, doubling time, and finite rate of increase. Mean generation time, net reproductive rate, doubling time, and finite rate of increase were analyzed by one-way ANOVA in PROC MIXED with host plants as independent fixed factors. Treatment means were separated by the Tukey-HSD test ($\alpha = 0.05$) (SAS 9.4, Institute Inc., Cary, NC, 2013). Age-specific survival graphs were plotted using SigmaPlot (SigmaPlot 14.0, Systat Software Inc., San Jose, CA, USA, 2018).

The EPG parameters recorded and the statistical test used for each parameter are listed in Table 1. Data analysis was performed using SAS software version 9.4 (SAS Institute Inc. Cary, NC, 2013). The effect of plant species on the proportion of individuals showing any certain EPG parameter was tested by a generalized linear model fitted in an exact logistic function. This method is more appropriate for small sample clustered binary data (Mehta and Patel 1995). On maize the proportion of individuals showing sustained feeding (Table 4.1, #18) and proportion of individual showing repetitive sieve element puncture (Table 4.1, #17) were very small causing complete separation, thus even with the use of exact logistic regression, this was leading to infinite estimates of logistic regression coefficients, therefore these maize parameters, as well as time from first salivation to first sustained feeding and number of sieve elements sustained

feeding events (Table 4.1, #23 and #26), had to be excluded of the analysis. Total time *M. sacchari* spent in a certain waveform during 4 h recording time were tested using one-way ANOVA in PROC GLM.

Levenes's test for homogeneity of variances and Shapiro-Wilk to test normality of data were performed previous to ANOVA. In case of non-normality of the errors or inequality of variances (Total cell punctures, Total duration of sieve element salivation, Total duration of pathway, Table 4.1, #10, #27 an #15) Welch's' ANOVA was applied. Tukey-HSD at 5% of significance was used to find out which means were different. Generalized linear model with Poisson response and link log was used to test the difference for counted EPG parameters (Table 4.1).

Table 4.1. Variables and test statistics used on EPG parameters of *M. sacchari* on different plant species. SE = sieve elements.

EPG Parameter	Statistical Test Applied
1. Proportion of individuals starting penetration	GLM-Logistic
2. Number of probes	POISSON
3. Total penetration time	ANOVA
4. Non-penetration time	ANOVA
5. Time from start of experiment to probe	ANOVA
6. First probe duration	ANOVA
7. Second probe duration	ANOVA
8. Number of probes shorter than 30s	GLM - Poisson
9. Number of probes shorter than 3 min	GLM - Poisson
10. Total cell punctures	POISSON
11. Cell puncture frequency per minute	ANOVA
12. Cell punctures per probe	ANOVA
13. Number of probes before first SE salivation or feeding	GLM - Poisson
14. Number of cell punctures before first SE salivation or feeding	GLM - Poisson
15. Total duration of pathway phase	ANOVA
16. Proportion of individuals showing SE phase	GLM-Logistic
17. Proportion of individuals showing repetitive punctures on SE	GLM-Logistic
18. Proportion of individuals showing sustained SE feeding	GLM-Logistic
19. Time from start of experiment to first SE salivation	ANOVA
20. Time from start of experiment to first SE feeding	ANOVA
21. Time from first probe to first SE salivation	ANOVA
22. Time from first probe to first SE feeding	ANOVA
23. Time from first salivation to sustained feeding	ANOVA
24. Number of SE salivation events	GLM - Poisson
25. Number of SE feeding events	GLM - Poisson
26. Number of SE sustained feeding events	GLM - Poisson
27. Total duration of SE salivation	ANOVA
28. Total duration of SE feeding	ANOVA
29. Total duration of SE phase (salivation and feeding)	ANOVA
30. Proportion of individuals showing xylem ingestion	GLM-Logistic
31. Number of xylem ingestion	GLM - Poisson
32. Total duration of xylem ingestions	ANOVA

4.3. Results

Life table analysis. Life table analysis was assessed on sorghum, Johnsongrass, sugarcane, rice, wheat, maize and sweetpotato. In this no-choice study, *M. sacchari* larviposited on all host plants, however *M. sacchari* only developed to adult on sorghum, Johnsongrass, sugarcane, rice, and wheat. Age-specific survivorship (l_x) of *M. sacchari* confined on maize, Johnsongrass, sorghum, sugarcane, sweetpotato, rice, and wheat are shown in Fig. 4.1. *M. sacchari* reared on maize and sweetpotato died quickly (Fig. 4.1), indicating a Type III survivorship curve in which individuals initially have a rather low chance of survival. On wheat and rice, individuals had a relatively constant death rate, suggesting a Type II survivorship curve. On sorghum, sugarcane and Johnsongrass individuals survived well early in life, but older individuals death rates increased dramatically, data that clearly fits in a Type I survivorship curve (Fig. 4.1).

Melanaphis sacchari development time ranged from four to six days among host plants, with *M. sacchari* developing faster on sorghum and Johnsongrass ($F = 21.75, p < 0.0001$) (Table 2). Host plant affected the time between molts (stadium). Duration of the first instar ($F = 14.88, p < 0.0001$), second instar ($F = 7.47, p < 0.0001$), and fourth instar ($F = 6.84, p < 0.0001$) were significantly different among plant species (Table 2). *Melanaphis sacchari* spent the longest time as a first instar nymph on rice and the shortest second instar period on sweetpotato (Table 2). *Melanaphis sacchari* did not molt to the fourth instar on maize and only reached second instar on sweetpotato. Only 12% and 16% of *M. sacchari* completed development to adult on rice and wheat respectively (Table 4.2).

Reproductive parameters of *M. sacchari* were also affected by host plant. Mean reproductive time was significantly different among plant species ($F = 14.30, p < 0.0001$) (Table 4.2). *Melanaphis sacchari* remained reproductively active longer on sorghum and shortest on rice and wheat (Table 4.2). Plant host affected total progeny per female ($F = 30.02, p < 0.0001$), with the greatest production of nymphs per female on sorghum (Table 2). Progeny per female was also high on Johnsongrass (30.2) and sugarcane (28.0). Plant host also affected the mean production of progeny per day per female (m_x) ($F = 11.29, p < 0.0001$). Daily nymph production per female was lowest on rice and wheat (Table 4.2).

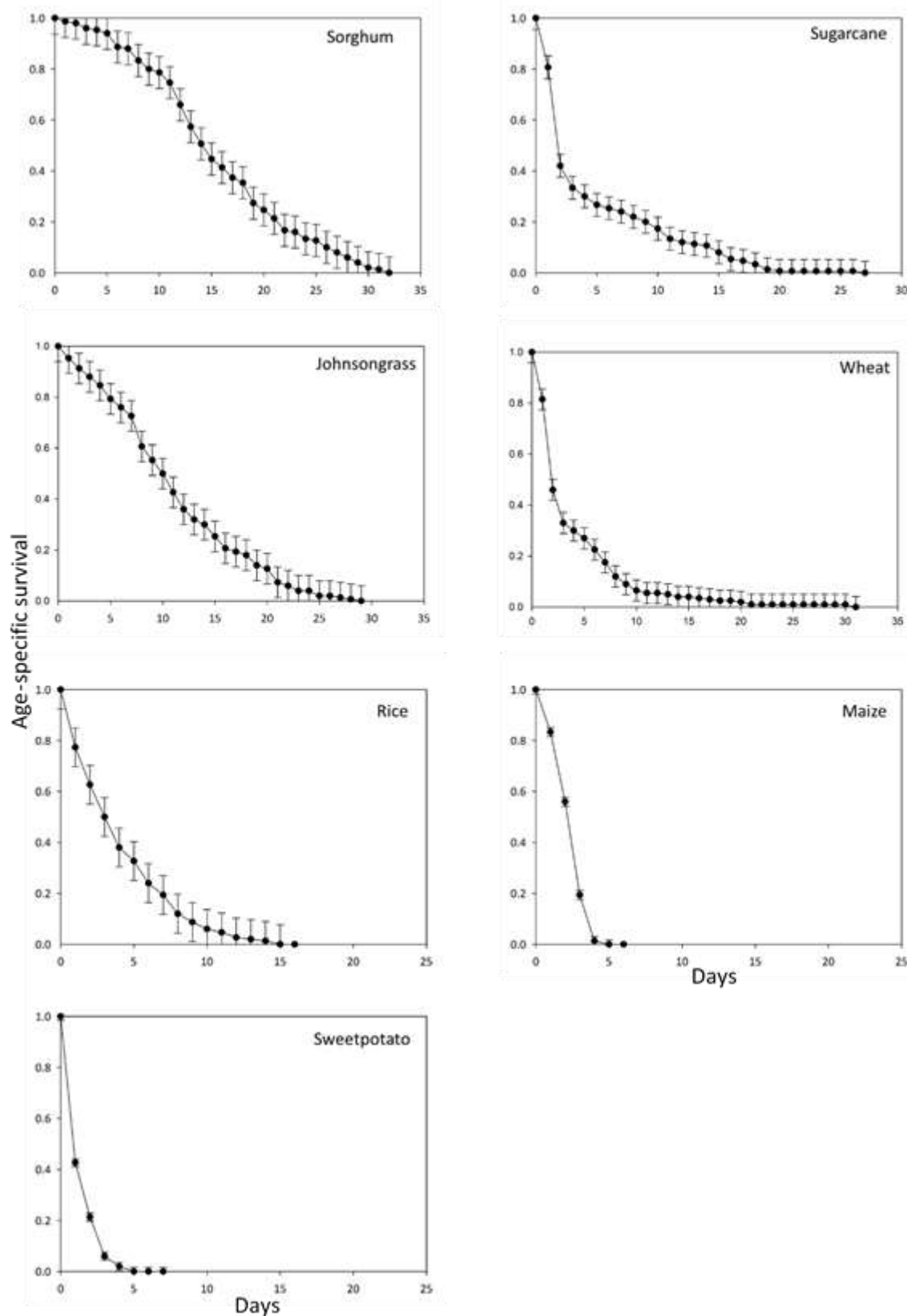


Figure 4.1. Age-specific survival of *M. sacchari* confined on different host plants.

Table 4.2. Biological variables of *M. sacchari* on different host plants under no-choice conditions. Values followed by different letters in the row are significantly different by the Tukey-HSD test ($\alpha = 0.05$).

Parameter	Plant Host						
	Sorghum	Johnsongrass	Sugarcane	Rice	Wheat	Corn	Sweetpotato
First Instar	1.3 ± 0.1 D	1.3 ± 0.1 D	1.4 ± 0.1 CD	3.0 ± 0.1 A	1.7 ± 0.1 AB	1.7 ± 0.10 BC	1.4 ± 0.1 CD
Second Instar	1.1 ± 0.1 AB	1.3 ± 0.1 A	0.9 ± 0.1 BC	1.5 ± 0.2 A	1.1 ± 0.1 AB	1.3 ± 0.1 A	0.8 ± 0.1 C
Third Instar	1.1 ± 0.1 A	1.2 ± 0.1 A	1.0 ± 0.1 A	1.2 ± 0.1 A	1.2 ± 0.1 A	1.0 ± 0.1 A	-
Fourth Instar	1.2 ± 0.1 B	1.3 ± 0.1 B	1.1 ± 0.2 B	0.8 ± 0.2 B	2.9 ± 0.2 A	-	-
Development Time (days)	4.0 ± 0.1 A	4.7 ± 0.1 A	5.0 ± 0.1 B	4.9 ± 0.2 AB	6.0 ± 0.1 C	-	-
Reproductive period (days)	9.4 ± 0.4 A	6.8 ± 0.4 B	6.8 ± 0.7 B	3.6 ± 0.9 C	5.4 ± 0.8 BC	-	-
Progeny per female	40.6 ± 1.7 A	30.2 ± 1.8 B	28.0 ± 3.0 B	5.9 ± 3.9 C	13.9 ± 3.9 C	-	-
Progeny per female per day (m_x)	4.4 ± 0.3 A	5.3 ± 0.3 A	4.1 ± 0.5 A	1.7 ± 0.61 B	2.6 ± 0.6 B	-	-

Intrinsic rate of increase, r_m , was affected by host plant (Table 4.3). *Melanaphis sacchari* reared on sorghum had the highest observed r_m followed by Johnsongrass, sugarcane, and wheat. Based on r_m , *M. sacchari* can't maintain population growth on rice (Table 4.3).

Net reproductive rate (R_0) also varied amongst hosts ($F = 3778.131$, $p < 0.0001$) (Table 4.3). *Melanaphis sacchari* on sorghum had the highest R_0 . *Melanaphis sacchari* mean generation time, T_G , was longest when reared on rice (11.7 days to complete one generation) and shortest on sorghum (8.6 days) ($F = 278.443$, $p < 0.00001$). *Melanaphis sacchari* population doubling time (DT) ($F = 24796.050$, $p < 0.0001$) and finite rate of increase (λ) ($F = 120213.985$, $p < 0.0000$) were lowest on rice, reaching negative DT values.

EPG. During the 4 h recording time, 50 to 78% of the 240 individuals probed on their respective test plants (Table 4.4). Plant species influenced the proportion of individuals starting stylet penetration ($F = 35.6$, $p < 0.0001$); maize had the lowest portion of individuals beginning penetration activities while sorghum and rice had the highest proportion of individuals starting penetration (Table 4.4).

Total time spent probing was highly variable (Table 4.4). For sorghum, the mean total stylet penetration duration was significantly longer than wheat or maize ($F = 6.74$, $p = <0.0001$). For *M. sacchari*, plant species did influence time to first probe (Table 4.4) ($F = 2.86$, $p = 0.0169$). *Melanaphis sacchari* averaged 255.5 s from the start of the experiment until first penetration on rice, 9-times shorter than on maize. Host plant also affected the number of probes per 4 hr (Table 4.4) with fewer probes on wheat and maize than on host plants ($X = 7.15$, $p < 0.0001$). The duration of first and second probes reflect the initial response of aphids to plants. The duration of the first probe was significantly shorter on rice (approximately 270 s) than on the other plants

(Table 4) ($F = 2.92$, $p = 0.0151$), but host plant did not affect the duration of the second probe
(Table 4) ($F = 0.57$, $p = 0.7235$).

Table 4.3. Population parameters (mean \pm SE) of *M. sacchari* confined on sorghum, Johnsongrass, sugarcane, rice, wheat, maize and sweetpotato

Parameter	Plant Host				
	Sorghum	Johnsongrass	Sugarcane	Rice	Wheat
Intrinsic rate of increase (r_m)	0.466 \pm 0.008	0.289 \pm 0.017	0.213 \pm 0.047	-0.02 \pm 0.027	0.044 \pm 0.032
Net Reproductive rate (R_0)	54.3 \pm 0.01 A	15.9 \pm 0.01 B	6.9 \pm 0.01 C	0.9 \pm 0.03 E	1.6 \pm 0.03 D
Mean Generation Time (T_G)	8.6 \pm 0.001 D	9.9 \pm 0.002 B	9.1 \pm 0.006 C	11.7 \pm 0.055 A	10.8 \pm 0.007 A
Doubling time (DT)	1.5 \pm 0.0002 D	2.4 \pm 0.0094 C	2.7 \pm 0.0056 B	-34.6 \pm 0.2707 E	15.8 \pm 0.0071 A
Finite rate of increase (λ)	1.6 \pm 0.0009 A	1.3 \pm 0.0001 B	1.2 \pm 0.0004 C	0.9 \pm 0.0002 E	1.0 \pm 0.0002 D

Table 4.4. EPG parameters of *M. sacchari* on different host plants. SE = sieve elements. Means (\pm standard error) followed by different letters on the row are different at 5% of significance level.

EPG parameter	Host Plant					
	Sorghum	Johnsongrass	Rice	Wheat	Maize	Sweetpotato
1. Proportion of individuals starting penetration	0.8 A	0.7 AB	0.8 A	0.7 AB	0.5 B	0.7 AB
2. Number of probes	8.3 \pm 0.9 A	5.4 \pm 0.7 AB	9.5 \pm 1.1 A	4.6 \pm 0.8 B	3.6 \pm 0.7 B	9.6 \pm 1.2 A
3. Total penetration time	9440.6 \pm 757.1 A	6472.6 \pm 787.7 ABC	6409.3 \pm 777.3 ABC	3224.3 \pm 796.3 C	4912.8 \pm 1117.1 BC	7203.3 \pm 803.6 AB
4. Non-penetration time	4921.0 \pm 716.0 C	8146.5 \pm 784.5 ABC	8044.9 \pm 773.2 BC	11441.7 \pm 797.0 A	9753.2 \pm 1117.1 AB	7462.6 \pm 803.6 C
5. Time from start of experiment to probe	1483.5 \pm 477.8 AB	3304.7 \pm 734.9 A	1110.5 \pm 368.6 BC	1623.2 \pm 566.2 AB	3143.6 \pm 803.8 AB	1429.2 \pm 503.8 AB
6. First probe duration	975.1 \pm 404.3 AB	1651.8 \pm 497.2 AB	255.5 \pm 109.2 BC	544.4 \pm 566.2 AB	2336.1 \pm 813.8 A	809.6 \pm 410.8 B
7. Second probe duration	1426.0 \pm 546.6 A	953.9 \pm 337.7 A	786.4 \pm 331.5 A	584.6 \pm 259.6 A	1348.4 \pm 677.7 A	871.5 \pm 354.8 A
8. Number of probes shorter than 30s	2.3 \pm 0.4 A	1.41 \pm 0.3 AB	3.2 \pm 0.6 A	1.0 \pm 0.2 B	1.3 \pm 0.5 AB	2.7 \pm 0.6 A
9. Number of probes shorter than 3min	3.3 \pm 0.5 AB	2.5 \pm 0.5 B	5.3 \pm 0.8 A	2.7 \pm 0.5 B	1.89 \pm 0.7 B	4.7 \pm 0.8 A
10. Total cell punctures	92.4 \pm 10.2 A	68.3 \pm 11.6 A	61.0 \pm 10.5 A	16.8 \pm 3.3 B	39.2 \pm 9.6 A	57.6 \pm 9.8 A
11. Cell puncture frequency per minute	0.7 \pm 0.1 A	0.7 \pm 0.1 A	0.5 \pm 0.1 A	0.5 \pm 0.1 A	0.6 \pm 0.1 A	0.6 \pm 0.1 A
12. Cell punctures per probe	13.7 \pm 1.7 A	13.2 \pm 2.0 A	8.0 \pm 2.1 AB	4.5 \pm 0.8 B	16.4 \pm 3.9 A	5.6 \pm 0.7 B
13. Number of probes before first SE contact	3.6 \pm 0.7 AB	1.9 \pm 0.4 B	7.4 \pm 1.6 A	2.9 \pm 0.8 B	1.00 \pm 0.4 C	-

(Table cont`d)

Table 4.4. Continued. EPG parameters of *M. sacchari* on different host plants. SE = sieve elements. Means (\pm standard error) followed by different letters on the row are different at 5% of significance level.

EPG parameter	Host Plant					
	Sorghum	Johnsongrass	Rice	Wheat	Maize	Sweetpotato
14. Number of cell punctures before first SE contact	44.2 \pm 6.1 A	36.3 \pm 7.7 A	47.4 \pm 12.4 A	20.3 \pm 3.8 A	43.6 \pm 7.7 A	-
15. Total duration of pathway phase	6723.9 \pm 690.2 A	5238.6 \pm 584.4 AB	4568.8 \pm 671.6 AB	2326.1 \pm 402.8 B	4160.1 \pm 747.1 AB	-
16. Proportion of individuals showing SE phase	0.8 A	0.7 AB	0.45 BC	0.3 CD	0.2 D	-
17. Proportion of individuals showing repetitive punctures on SE	0.9 A	0.6 AB	0.36 B	0.3 B	-*	-
18. Proportion of individuals showing sustained SE feeding	0.6 A	0.9 A	0.71 A	0.9 A	-*	-
19. Time from start of experiment to first SE salivation	5643.1 \pm 625.4 A	5442.6 \pm 869.8 A	6811.6 \pm 1118.4 A	6127.6 \pm 1382.9 A	6098.5 \pm 1850.2 A	-
20. Time from start of experiment to first SE feeding	5191.2 \pm 606.6 A	5794.3 \pm 886.7 A	8012.91 \pm 1131.9 A	6159.1 \pm 1379.9 A	6886.5 \pm 1961.3 A	-
21. Time from first probe to first SE salivation	4590.2 \pm 619.2 AB	2512.5 \pm 475.8 B	6801.1 \pm 997.2 A	5072.7 \pm 235.6 AB	2951.5 \pm 1005.4 AB	-

(Table cont'd)

Table 4.4. Continued. EPG parameters of *M. sacchari* on different host plants. SE = sieve elements. Means (\pm standard error) followed by different letters on the row are different at 5% of significance level.

EPG parameter	Host Plant					
	Sorghum	Johnsongrass	Rice	Wheat	Maize	Sweetpotato
22. Time from first probe to first SE feeding	4840.1 \pm 583.9 AB	2864.1 \pm 583.9 B	7684.3 \pm 583.9 A	5104.1 \pm 583.9 AB	3440.2 \pm 1083.8 B	-
23. Time from first salivation to first sustained feeding	1631.0 \pm 743.5 A	315.5 \pm 264.1 A	1355.4 \pm 710.2 A	30.9 \pm 8.9 B	_*	-
24. Number of SE salivation events	3.3 \pm 0.5 A	2.5 \pm 0.3 A	2.2 \pm 0.3 AB	1.3 \pm 0.2 B	3.25 \pm 0.9 A	-
25. Number of SE feeding events	1.7 \pm 0.3 A	1.9 \pm 0.2 A	1.8 \pm 0.2 A	1.4 \pm 0.2 A	2.0 \pm 0.5 A	-
26. Number of SE sustained feeding events	0.8 \pm 0.1 A	1.3 \pm 0.2 A	1.2 \pm 0.2 A	1.1 \pm 0.3 A	_*	-
27. Duration of SE salivation	82.8 \pm 10.8 A	57.4 \pm 9.6 B	3.9 \pm 30.2 D	35.6 \pm 9.4 C	51.6 \pm 8.2 B	-
28. Duration of SE feeding	3236.4 \pm 710.6 A	4743.2 \pm 1956.1 A	3718.5 \pm 790.9 A	4205.9 \pm 1562.6 A	3056.3 \pm 1668.7 A	-
29. Duration of SE phase	3315.9 \pm 712.1 A	4800.5 \pm 1963.1 A	3832.5 \pm 784.6 A	4241.5 \pm 1567.8 A	5199.9 \pm 1924.7 A	-
30. Proportion of individuals showing xylem ingestion	0.10 A	0.2 A	0.16 A	0.22 A	0.20 A	0.26 A
31. Number of xylem ingestion	1.0 \pm 0.6 A	0.4 \pm 0.2 AB	0.2 \pm 0.1 B	0.3 \pm 0.1 B	0.5 \pm 0.3 AB	0.63 \pm 0.3 A
32. Total duration of xylem ingestions	1242.0 \pm 834.4 A	1116.5 \pm 635.1 A	3066.7 \pm 1118.1 A	2595.0 \pm 2038.2 A	4460.4 \pm 951.3 A	4919.5 \pm 1282.7 A

* Corn samples were removed from the analysis, see Material and Methods section.

Aphids penetrate the plant tissue only briefly with very short probes of 30 sec called sap sampling in order to determine host suitability (Table 4.4). *Melanaphis sacchari* made twice as many short probes on sorghum, rice, and sweetpotato than on wheat ($X = 22.80$, $p < 0.0001$). *Melanaphis sacchari* made significantly more probes longer than 30 s but shorter than 3 min on rice and sweetpotato ($X = 21.20$, $p = 0.0007$).

Sheath salivation, cell-puncturing, and stylet bundle movement towards the sieve elements characterize the pathway phase, excluding xylem phase and all SE-related phases (Table 4.4) (Tjallingii and Esch 1993). *Melanaphis sacchari* on sorghum spent almost 3-times longer in the pathway phase than they did on wheat ($X = 22.80$, $p < 0.0001$) ($F = 4.67$, $p = 0.0022$). Along the pathway phase, *M. sacchari* punctured the adjacent cells (Tjallingii and Esch 1993). The total number of cell punctures per 4 hr of recording (Table 4.4) was significantly lower on wheat than on the other plant species ($F = 38.85$, $p < 0.0001$). The mean number of cell punctures per aphid probe (Table 4.4), was lower on wheat and sweetpotato than on the other host plants ($F = 6.21$, $p < 0.0001$). However, the frequency of cell punctures per minute (Table 4.4) was not different among the host plants ($F = 0.87$, $p = 0.5051$). Number of probes before reaching sieve elements (Table 4.4) was greater on rice than on other plants species ($F = 28.84$, $p < 0.0001$), but the number of cell punctures before reaching phloem (Table 4.4) was the same for all the plant species ($F = 5.29$, $p = 0.2588$).

The time from the start of the recording until first sieve element salivation (Table 4.4) ($F = 0.30$, $p = 0.8799$), and first sieve element feeding (Table 4) ($F = 1.29$, $p = 0.2838$) did not differ among host plants. However, when we controlled the non-activity time, time from first probe to first sieve element salivation (Table 4.4), and first sieve element feeding occurrence (Table 4.4),

we observed that *M. sacchari* took significant shorter time to salivate in sieve elements of Johnsongrass ($F = 4.71, p = 0.0021$) and longer time to the first feeding occurrence on rice ($F = 5.22, p = 0.0010$). No difference was observed on time from the first salivation to the first sustained feeding occurrence (Table 4.4) ($F = 1.29, p = 0.2846$).

The initial and global parameters associated with sieve elements are shown in Table 4. During repetitive sieve elements punctures, *M. sacchari* inserts its stylet repeatedly into the intracellular lumen of the sieve elements (Tjallingii and Gabrys 1999) (Table 4.4). Sixty percent and 85% of the individuals that reached phloem on sorghum and Johnsongrass plants showed repetitive sieve element puncture. On rice and wheat, these proportions were significantly lower (0–30%) ($\chi^2 = 18.4814, p < 0.0001$). Because only one individual on corn out of the 40 analysed showed this behavior, this individual was excluded from the analysis.

The proportion of individuals that initiated phloem phase differed among host plants (Table 4.4) ($\chi^2 = 72.0449, p > 0.0001$). On sorghum and Johnsongrass, 70 to 84% of individuals reached the sieve elements, while on wheat and rice significantly lower proportion of individuals (0–45%) reached this phase. Maize had the lowest proportion (0–20%) of individuals reaching phloem.

Melanaphis sacchari had the lowest number of salivation events on wheat ($\chi^2 = 13.38, p = 0.0096$) (Table 4.4). However, the number of feeding events (Table 4.4) was the same on all the host plants during the 4 hr ($\chi^2 = 1.18, p = 0.8814$). Also, sugarcane aphid spent longer time salivating in sorghum sieve elements than other plants, salivating 21X longer sorghum than in rice (Table 4) ($F = 3.58, p = 0.0197$).

The proportion of individuals showing sustained feeding, defined as feeding activity > 10min (Table 4) ($\chi^2 = 7.1794$, $p = 0.1267$), the number of sustained feeding events per 4 hours of recording (Table 4) ($F = 3.64$, $p = 0.4567$), the total time feeding on the sieve elements during the 4 hr recording (Table 4) ($F = 0.27$, $p = 0.8955$), and the total time spent on phloem phase per 4 hr (Table 4, #29) ($F = 0.29$, $p = 0.8821$) did not differ among any aphid-plant combination. On maize, only two individuals out of forty achieved sustained feeding, so to avoid infinite parameter estimates in the logistic regression these two aphids had to be removed from the analysis.

The proportion of individuals showing xylem ingestion (Table 4.4) was not different among the host plants ($\chi^2 = 3.5098$, $p = 0.6219$). The number of xylem ingestion events per 4 hr of recording (Table 4.4) was most significant on sweetpotato and sorghum ($F = 11.94$, $p = 0.0356$). However, even though the amount of time spent in xylem per 4 hr was 4.4-times greater on sweetpotato than on Johnsongrass, we did not detect significant differences among the host plants (Table 4.4) ($F = 1.54$, $p = 0.2141$).

4.4. Discussion

This study provides new information regarding *M. sacchari* biology and behavior and its ability to colonize agronomic crops common to Louisiana. Colonizing aphids are those aphid species that settle and reproduce on the host (Wosula et al. 2013). *Melanaphis sacchari* did colonize sorghum, Johnsongrass, sugarcane, rice, and wheat in the laboratory. Although there are reports of *M. sacchari* being able to transmit Sugarcane yellow leaf virus, a restricted phloem virus, to rice and wheat (Schenck and Lehrer 2000), this study is the first to demonstrate that *M.*

sacchari could develop on rice and wheat and the first to describe, in detail, *M. sacchari* feeding behavior on these host plants. *Melanaphis sacchari* can use rice as a host and can successfully colonize wheat, sugarcane, and Johnsongrass, and when sorghum is not available, from September through May, these plants may serve as alternative hosts to *M. sacchari*. The constant supply of plants species in which this *M. sacchari* genotype can feed may be one factor contributing to the sorghum outbreaks.

Melanaphis sacchari on sorghum and Johnsongrass developed the fastest and had the greatest intrinsic rate of increase. In the United States, Johnsongrass is a highly invasive species, known to be broadly distributed and frequently found associated or around sorghum fields (Ohadi et al. 2018). It is a perennial *Sorghum* species that produce tillers and rhizomes, which promotes fast aerial growing (Cox et al. 2018). In southern states, Johnsongrass is present throughout the winter months, and when temperatures start to rise, Johnsongrass is available before sorghum (Bowling et al. 2016). In field conditions, *M. sacchari* populations can rapidly increase early in the season on Johnsongrass and subsequently move to sorghum fields. Insect species present in the field or very close surroundings have an advantage over other species that must still invade the crop. In this case, colonization of the crop is almost immediate, and the population density at any part of the field will increase fast (Price and Waldbauer 1994). The massive population densities seen on sorghum fields are likely a consequence of the abundant Johnsongrass presence, and according to our results, areas planting sorghum will require thoughtful weed management in the field as well as in the surroundings due not only to competition with Johnsongrass but also as an excellent host for this insect pest.

In contrast with Johnsongrass, *M. sacchari* from sugarcane, wheat, and rice will migrate and sorghum fields will be colonized gradually. This movement between crops can be triggered by distinct phenologies, as when the potato leafhopper, *Empoasca fabae* Harris, are driven to nearby soybean fields when alfalfa is cut (Price and Waldbauer 1994). We demonstrate that there is the potential for multiple crop use within the agroecoscape by *M. sacchari* which could facilitate migration between sorghum, sugarcane, wheat, and rice system. Therefore planning around the spatial relationship of sorghum, sugarcane, wheat, and rice should be done at the ecosystem level in anticipation of *M. sacchari* movement. In this way, intense colonizing populations may be avoided or at least predicted (Price and Waldbauer 1994).

While Lopes-da-Silva et al. (2014) did not find differences in *M. sacchari* time to first reproduction between sorghum and sugarcane, our study found that development was the fastest on sorghum. Shorter development time is considered the better growth strategy for insects (Roitberg et al. 2001). Besides the decrease in predation and parasitism, shorter development increases insect reproductive performance (Ives and Settle 1997, Chau and Mackauer 2001, Harvey and Standart 2002). In terms of pest management, this study indicates that in sorghum fields *M. sacchari* migrants would establish colonies earlier than on sugarcane fields.

Another integral component of r_m is aphid fecundity. *Melanaphis sacchari* highest nymphal production of 40 nymphs per female was lower than rates observed by van Rensburg (1973) (85.6 nymphs per female) and Abe et al. (2011) (80 nymphs per female) but greater than the production of 25.6 nymphs per female observed on sorghum by Lopes-da-Silva et al. (2014). Variances in experimental conditions and varieties might explain fertility differences on sorghum.

While there is no information on which sorghum variety or plant stage Abe et al. (2011) used, van Rensburg (1973) used grain sorghum plants at the four-leaf stage, and Lopes-da-Silva et al. (2014) tested sweet-sorghum (var. BRS506), using aphids from an isofemale lineage collected on sugarcane field.

Melanaphis sacchari fecundity on sorghum was the highest. Similarly, Lopes-da-Silva et al. (2014) found that *M. sacchari* on sorghum produced significantly more nymphs than on sugarcane. *Melanaphis sacchari* production of 28 nymphs per female on sugarcane is higher than the range of nymph production on sugarcane found in the literature, which varies from 2.1 to 3.4 to (Lopes-da-Silva et al. 2014, Akbar et al. 2010, Fartek et al. 2012). The intrinsic rate of increase on sugarcane of 0.213 was much higher than the range of -0.094 to 0.158 found in other studies (Akbar et al. 2010, Fartek et al. 2012).

In the present work, this *M. sacchari* clone, collected and maintained on sorghum plants in Louisiana, performed better, in terms of biological variables and population parameters, on sugarcane than the *M. sacchari* collected and kept on sugarcane used by Akbar et al. (2010), also in Louisiana. In Louisiana, sugarcane is harvested in late-October to late-November, and as perennial grass, it starts to sprout as soon as the soil heats up. Even though the *M. sacchari* clone previously associate with sugarcane is still found in Louisiana (Nibouche et al. 2018), the sorghum-associate clone appears to increase its population faster than the clone used in Akbar et al. (2010) experiments, and therefore more frequent and larger populations of sugarcane aphid may be found in sugarcane in the future. This will likely shift the need to monitor and control this insect earlier in the crop season, increasing farmer costs.

Overall, parameters to estimate population growth (r_m , R_0 , λ) were the lowest on rice and the highest on sorghum plants. Several studies show the correlation between aphid biological traits and feeding behavior (Fartek et al. 2012, Akbar et al. 2014, Davis and Radcliffe 2008). Likewise *M. sacchari* life table parameters were associated with its feeding behavior on each plant.

Melanaphis sacchari could not develop on maize and sweetpotato plants, and the larviposited nymphs had low age-specific survival and longevity, which was a consequence of starvation because *M. sacchari* did not show sustained feeding on these plants. On sweetpotato, none of the individuals of *M. sacchari* were able to reach the sieve elements during the 4-hour EPG recording. On maize, 20 percent of the individuals that initiate activity reached phloem. The lowered life table parameters observed in this work were a consequence of *M. sacchari* starvation on these plants.

External and internal factors could have deterred *M. sacchari* feeding and development on these plants (Dreyer and Campbell 1987, Givovich and Niemeyer 1995, Powell et al. 1999), and since *M. sacchari* started to probe and navigated through plant tissues, we suggest that epidermal and mesophyll plant factors prevented it from reaching phloem. Plant epidermal volatile compounds can interfere with aphid feeding. Constitutive rice emissions of (E)- β -farnesene and limonene played a crucial role in rice repellence against *Rhopalosiphum padi* L. (Sun et al. 2017). *Aphis fabae* Scop. when exposed to the oat extract of 1-hexacosanol quickly leaves the plant (Powell et al. 1999).

Melanaphis sacchari may also be reacting to compounds present in the plant mesophyll. Naturally occurring flavonoids and polar phenolic fractions showed the highest deterrence

activity against *Myzus persicae* Sulzer (green peach aphid) and *Schizaphis graminum* Rondani (greenbug) on wheat plants (Dreyer and Jones 1981). Different levels of DIMBOA, the main hydroxamic acid isolated from maize, produced deleterious effects on *S. graminum* due to feeding deterrence and toxicity (Argandona et al. 1983).

Aphids reached the xylem on all host plants, but the greatest number of xylem ingestion events was detected on sorghum and sweetpotato. Studies have demonstrated that aphids feeding on phloem also drink xylem sap (Spiller et al. 1990, Powell and Hardie 2002, Daniels et al. 2009). Spiller et al. (1990), which first observed this behavior, proposed that the water deficiency, generated in the body due to the high concentration of gut contents, is restored and maintained by xylem ingestion. Xylem ingestion is also correlated with periods of starvation in aphids (Spiller et al. 1990, Ramírez and Niemeyer 2000, Daniels et al. 2009). Thus, on sorghum the high sap ingestion, and on sweetpotato the inability to reach the sieve elements explains the increased number of xylem ingestion.

On rice and wheat, 45 and 30% of the individuals reached phloem, respectively, while on sorghum and Johnsongrass many individuals (71–85%) reached this phase. Aphids on rice and wheat readily started probing activities once placed on the plants. Wheat showed the highest non-penetrating total time, and the lowest number of probes during the experiment, thus *M. sacchari* might be reacting to epicuticular lipids of wheat plants (Storer et al. 1996, Powell et al. 2006, Pettersson et al. 2007). However, the response to wheat surface and epidermal factors appears to be switched off after a while, and other factors seem to be preventing contact with sieve elements (Storer et al. 1996, Powell et al. 2006, Pettersson et al. 2007). On wheat, once *M. sacchari* located phloem, it salivated briefly and then started to feed. Similarly, Davis and

Radcliffe (2008) observed that *M. persicae* had significantly longer nonpenetration duration on winter wheat.

This project was the first to show sugarcane aphid feeding on wheat and rice. The fact that *M. sacchari* reached the phloem and started to feed on wheat and rice corroborates with observations of transmission of sugarcane yellow leaf virus (ScYLV) (family Luteoviridae, genus Polerovirus), a phloem restricted virus infecting wheat and rice as well as sugarcane (Schenck and Leher 2000). Our study indicates that *M. sacchari* can use wheat and rice as bridging species to maintain populations while sorghum is not available, and although the population growth is low, it could also establish sustained phloem feeding (>10 min feeding) on wheat and rice. Because *M. sacchari* can transmit ScYLV to wheat and rice (Scheneck and Leher 2000), and because sugarcane producing areas are also wheat and rice producers (USDA, NASS 2018), there is the potential for increased ScYLV spread to sugarcane fields.

Chemical factors usually play the primary role in inducing negative feeding behavior on aphids (Dreyer and Jones 1981, Kharbangar et al. 2015). In wheat and rice, both DIMBOA and phenolic compounds (mainly flavonoids), act as feeding deterrents (Dreyer and Jones 1981, Givovich and Niemeyer 1995, Kharbangar et al. 2015). For instance, wheat seedlings with higher hydroxamic acid concentrations increased time to reach phloem of *R. padi*, *S. graminum*, *Sitobion avenae* Fabricius, and *Metopolophium dirhodum* Walker (Givovich and Niemeyer 1995). Instead of DIMBOA, the major aphid feeding deterrents and toxic compounds in sorghum are dhurrin, P-hydroxybenzaldehyde (phenol), and procyanidin (condensed tannin) (Dreyer and Jones 1981, Dreyer et al. 1981). These compounds are known to cause aphid deterrence (Dreyer et al. 1981, Leszczyński et al. 1985) and toxicity (Grayer et al. 1992, Pettersson 1994, Kaur et al. 2017).

From the upper mesophyll, *M. sacchari* navigated its stylets through the extracellular space toward the sieve elements. On sorghum, *M. sacchari* spent about 7,320 s in pathway phase, which is comparable with the 4,200-6,000 minutes spent in pathway phase on susceptible sugarcane varieties (Akbar et al. 2010, Fartek et al. 2012).

A higher percentage of aphids (60 to 85%) repetitively punctured sieve elements on sorghum and Johnsongrass than on rice and wheat (25 to 36%). Similarly, in pea aphid the proportion of individuals carrying this behavior on less suitable or non-host plants was significantly lower (10–25%) than on native and universal hosts (65–100%) (Schwarzkopf et al. 2013), and feeding periods of *Brevicoryne brassicae* L. on its host plant *Sinapis alba* were mostly preceded by repetitive sieve element punctures (Tjallingii and Gabrys 1999).

The high percentage of aphids that repetitively punctured sieve elements on sorghum and Johnsongrass, coupled with the greater fitness on these plants, indicates that this behavior is linked to successful *M. sacchari* host plant exploitation. Sieve element punctures, which include salivation, might be conditioning sorghum and Johnsongrass plants for subsequent feeding. Salivary compounds, such as Ca^{2+} -binding molecules, can suppress sieve element occlusion (Bos et al. 2010, Atamian et al. 2013, Elzinga and Jander 2013, Pitino and Hogenhout 2013, Medina-Ortega and Walker 2013). The role of salivation in aphid-plant interactions appears to be aphid-plant specific (Medina-Ortega and Walker 2013). In fact, Pitino and Hogenhout (2013) found that specific salivary effectors of *M. persicae* produced different performance responses in a plant-specific way. Thus, it is likely that *M. sacchari* is modulating sorghum and Johnsongrass responses to infestation, and the repetitive puncture behavior coupled with longer E1 time to establish feeding is aiding the successful use of *Sorghum spp.* by *M. sacchari*.

After the repetitive punctures on sieve elements, aphids started to salivate into the sieve elements. Phloem activity is characterized by waveforms E1 (watery salivation on sieve element) and E2 (sap ingestion and salivation). Watery saliva secretion is an essential component of aphid feeding, and it always precedes sap ingestion (Prado and Tjallingii 1994). Salivation into sieve elements plays a vital role in overcoming the defense mechanisms of plants by conditioning a plant for feeding.

Melanaphis sacchari had the longest watery saliva injection into the phloem lumen (E1 phase) on sorghum and Johnsongrass than in the other plants. Similarly, Fartek et al. (2012) observed that *M. sacchari* salivated 1.5-times longer on the sugarcane variety that promoted the highest population increase, and for *M. persicae*, sieve element salivation was the longest on potato, which was also the most suitable plant for aphid development (Davis and Radcliffe 2008).

However *M. sacchari* repetitive puncturing or salivation did not interfere with *M. sacchari* feeding time on the other plants. In fact, once *M. sacchari* reaches the sieve elements, it establishes sustained feeding regardless of the host plant. Phloem ingestion has been regarded as host plant acceptance with respect to feeding (Montllor and Tjallingii 1989, Powell 2006, Pettersson et al. 2007). However, in this study, phloem acceptance achieved in the different host plants resulted in very different population growth parameters, such as the negative intrinsic rate of increase on rice plants. This shows that for *M. sacchari*, host plant feeding does not correlate with host plant colonization, in which a population can establish and multiply (Wosula et al. 2013, Hancock et al. 2015).

The fact that *M. sacchari* fitness was greatly different amongst the host plants, while being able to reach and feed on sieve elements at the same rates, points to sieve element located

factors affecting aphid fitness on wheat and rice. Similarly, Schwarzkopf et al. (2013) observed that the poor performance of pea aphid clones on less suitable and non-host plants was mainly due to sieve elements located factors, and Ponder et al. (2000) showed that sieve elements factors explain the lowered performance of *R. padi* on nitrogen deficient barley.

Nutritional incompatibility is the first explanation that comes to mind for *M. sacchari* exhibiting the same feeding rates on sorghum, Johnsongrass, rice, and wheat with extreme variations in aphid fitness. In aphids, the feeding rate is determined by sucrose concentration (Mittler and Meikle 1991, Douglas 2006), and dietary sucrose concentration is so important for sustained feeding that at concentrations below 0.2 M aphids are not able to establish sustained feeding (Pescod et al. 2007). The proportion of individuals showing sustained feeding as well as in total time feeding were the same among host plants, indicating that in all plants the sucrose concentration was appropriate.

Contrary to lipids and sterols, aphids have a high dietary nitrogen demand. It is well known that concentration and composition of dietary amino acids affects aphid performance (Mittler and Dadd 1962, Mittler 1967, Mittler 1970, Srivastava and Auclair 1975, Prosser et al. 1992, Ponder et al. 2000). Amino acids are the dominant nitrogenous compounds on phloem sap, and it is the primary nitrogenous compound used by aphids. In rice, amino acids constitute 3 to 8% of the phloem (w/v) and on wheat 26%, mostly present in a free form (Fukumorita and Chino 1982, Hayashi and Chino 1986). The composition of the phloem sap of sorghum and Johnsongrass is not known, but on rice phloem sap cysteine and methionine are present in negligible amounts (Fukumorita and Chino 1982), while on wheat, butyric acid is absent (Hayashi and Chino 1986).

Investigating *M. sacchari* feeding behavior on sugarcane cultivars HoCP 91-555 (resistant), LCP 85-384 (resistant), and L 97-128 (susceptible), Akbar et al. (2014) observed that cultivar effects were not detected for levels of total phenolics, available carbohydrates, and water potential, but two free essential amino acids, histidine and arginine, were absent from phloem sap in HoCP 91-555. Two free essential amino acids, leucine and isoleucine, and two free non-essential amino acids, tyrosine and proline, were absent from honeydew of aphids fed on HoCP 91-555. Aphids feeding time was 2-times lower on this resistant cultivar than on the susceptible one, and the authors suggest that the inability of *M. sacchari* and its primary endosymbionts (e.g., *Buchnera*) to synthesize the amino acids was the cause for the decreasing in feeding time and consequently reduced growth potential of *M. sacchari* on HoCP 91-555 (Akbar et al. 2014). Therefore, *M. sacchari* growth is indeed affected by the amino acid compositions of plant sap. Hence, the reduced population growth of *M. sacchari* on rice and wheat in the present study suggest that the cysteine, methionine and butyric acid lacking on rice and wheat are essential for *M. sacchari* population increase.

On wheat the plant phloem based defense is based on the sieve occlusion by phloem lectins, which in turn are regulated by MYB transcription factors and ethylene signaling (Zhai et al. 2017). Thus since the defense response of wheat have an additional route of expression, *M. sacchari* may not be able to inhibit the ethylene response on these plants, resulting in its lowered life table and population parameters.

In general, for this *M. sacchari* clone infesting sorghum in the United States, we can conclude that epidermal and mesophyll factors (volatile and deterrent compounds) are important in initial rejection of maize and sweetpotato. Difficulties in locating and accepting

phloem sap combined with low nutrition quality and phloem located plant defense (sieve elements-factors) explain the lowered fitness of *M. sacchari* on rice and wheat. High fitness on sorghum and Johnsongrass is associated with *M. sacchari* effectiveness to reach and feed on the sieve elements, as well as the nutrient supply these plants provide. Additionally *M. sacchari*'s ability to manipulate plant physiology and metabolism through sieve element punctures and salivation may also play an important role in the successful aphid performance. This connection between host selection behavior and physiology provides a strong basis for this *M. sacchari* to be considered a superclone (Vorburger et al. 2003, Chen et al. 2013, Harrison and Mondor 2011).

These findings have important use in the understanding of *M. sacchari* sorghum colonization. Likely *M. sacchari* is using wheat plants to maintain a low population level after the harvesting of sorghum, then the migrants can use rice, maize as a source of hydration or bridging plants while seeking for more suitable hosts.

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CHAPTER 5. CHARACTERIZATION of LEVELS of RESISTANCE to *Melanaphis sacchari* in SORGHUM PLANT INTRODUCTION (PI) GENOTYPES

5. 1 Introduction

Melanaphis sacchari (Zehnter) (sugarcane aphid) is an economically important pest of sugarcane worldwide (Blackman and Eastop 2000, Singh et al. 2004, White et al. 2001) and recently has become an important sorghum (*Sorghum bicolor* (L.) Moench ssp. *bicolor*) pest in the United States (Villanueva et al. 2014, Bowling et al. 2016, Brewer et al. 2017) where, in the past, it has been present only on sugarcane (*Saccharum officinarum* L.). The losses due to *M. sacchari* infestation on sugarcane (Hall 1987, White et al. 2001) are mainly due to the transmission of sugarcane yellow leaf virus (ScYLV), which is known to cause yield losses up to 50% (Vega et al. 1997, Schenck and Lehrer 2000, ElSayed 2015).

In 2013, the first outbreaks of *M. sacchari* on sorghum were reported in Beaumont, TX, and then in Louisiana, Oklahoma, and Mississippi (Villanueva et al. 2014, Bowling et al. 2016). In 2015, all areas producing sorghum in the United States reported yield losses due to *M. sacchari* infestation (Villanueva et al. 2014, Kerns et al. 2015, Bowling et al. 2016). In 2014, Louisiana and Mississippi, where 100% of the sorghum fields were infested, and 75% of the areas required chemical control, \$7.2 and \$3.5 million were spent, respectively, to control this pest (Brewer and Gordy 2016). To authenticate yield decline on sorghum hybrids and to measure aphid population growth, Brewer et al. (2017) conducted experiments at three southern United States grain sorghum production locations. In Louisiana and Texas, *M. sacchari* outbreaks corresponded with

a yield decline of up to 60% (Brewer et al. 2017). Plant sap removal and fungi growth on the leaves causes the yield losses (Bowling et al. 2016, Brewer et al. 2017).

Current management of *M. sacchari* relies on scouting followed by insecticide applications of Transform® (Dow AgroSciences, Sulfaxoflor (nicotinic acetylcholine receptor competitive modulators)) and Sivanto® prime (Bayer, Flupyradifurone (nicotinic acetylcholine receptor competitive modulators)) when economic thresholds are reached. Number of aphids per leaf or percentage of plants infested are being used. In Mississippi, Catchot et al. (2015) recommends starting insecticidal control when 20-30% of the field is infested with *M. sacchari*. In Arkansas, treatment is recommended when 25% of the plants are infested with more than 50 aphids per leaf (Seiter et al. 2015). For Louisiana, a preliminary threshold at the boot/milk stage of 50 aphids per leaf colonizing 20% of plants in the field is suggested by Brown et al. (2015). In Texas, Knutson et al. (2016) recommends sampling of 40 plants per field and initiating treatment according to costs of control, the market value of the grain, and stage of plant development.

Plant breeding for resistance is the more economically and safe method of protection from the pests, including aphids. Host plant resistance can reduce pest population levels and is compatible with biological control. Host plant resistance does not promote environment disequilibrium, has a cumulative and persistent effect, and does not require sophisticated technologies for utilization (Smith 2005, van Emden 2007, Stout and Davis 2009, Stout 2014).

Painter (1951) defined plant resistance to insects as the heritable traits affecting the level of damage caused by the insect, representing the capacity that certain plants possess to reach higher production than other plants under the same conditions. The classification of Painter (1951), modified by Kogan and Ortman (1978) categorizes plant resistance to insects in non-

preference, tolerance, and antibiosis, and this categorization is still a useful approach in host plant resistance studies.

Development of plant resistant varieties or cultivars traditionally involves four steps: screening, categorization, breeding, and implementation (Stout 2014). The first step consists of the evaluation of an array of plants with different genetic backgrounds, which can be lines, accessions, and cultivars, to detect resistance to a pest (Stout 2014). Screening for resistance for aphids depends on the type of resistance that one is interested in identifying.

Antixenosis is resistance to colonization by aphids through effects on behavior (van Emden 2007). Screening for antixenosis generally involves releasing alatae individuals over different plant genotypes and accessing their final choice on each genotype after periods of time (Cruz and Vendramim 1995, Starks et al. 1983, Teetes et al. 1974, Schweissing and Wilde 1979). However, for aphids, host plant selection involves probing the plant (stylet plant penetration with assessment of plant quality through the sensory system) (Tjallingii and Esch 1993, Pettersson et al. 2007). The electrical penetration graph (EPG) technique developed by McLean and Kinsey (1964, 1965, 1967) and improved subsequently (Tjallingii 1978, Tjallingii 1988, Kawabe and McLean 1978, Kawabe 1985, Kimsey and McLean 1987, Backus and Bennett 1992) enables the continuous monitoring of aphid probing activity, facilitating the understanding of host plant selection by aphids (Walker and Backus 2000).

Screening for antibiosis involves identifying whether or not the potential resistant plant negatively affects the population increase of an aphid species (van Emden 2007). The most common technique to study antibiosis resistance on aphids is with no-choice assays, in which aphids are confined individually or in groups (Dixon and Kindlmann 1990, Awmack and Leather

2007, van Emden 2007, Dixon 2012). In this method, individual survival, development time, and fecundity can be combined into populations growth estimations such as the intrinsic rate of increase (r_m) (Birch 1948, Awmack and Leather 2007), population doubling time and mean generation time (Birch 1942, Leather and Dixon 1984, Dixon and Kindlmann 1990). Using this method, sources of antibiosis resistance for aphids have been identified for important crops pests, such as *Rhopalosiphum padi* (L.), the bird cherry-oat aphid (Descamps and Chopra 2011, Taheri et al. 2010, Karami et al. 2016), *Sipha flava* (Forbes), the yellow sugarcane aphid (Pallipparambil et al. 2014), and *Schizaphis graminum* (Rondani), greenbug (Nuessly et al. 2008, Vakhide and Safavi 2014).

Additionally, the categories of resistance can be subdivided into induced or constitutive, direct or indirect (Stout 2013). Induced resistant traits are traits only expressed (or more strongly expressed) after detection of injury by the plant. In constitutive resistance, the plant continually expresses traits of resistance. Direct resistant traits affect either the biology or behavior of the insect itself, while indirect resistance traits affect natural enemies (Stout 2013).

However, data on *M. sacchari* resistance in sorghum is scarce. Only a few hybrids and genotypes express some level of resistance (Teetes et al. 1995, Sharma et al. 2013, Sharma et al. 2014, Armstrong et al. 2015, Brewer et al. 2017, Szczepaniec 2018) effective against *M. sacchari*.

From an initial screening of 462 sorghum lines in Botswana and Zimbabwe, Teetes et al. (1995) selected 34 lines that were further evaluated in greenhouse tests, after which the authors found 12 lines expressing antibiosis resistance to *M. sacchari* (Teetes et al. 1995). In India, Sharma et al. (2013) tested 31 sorghum genotypes for *M. sacchari* resistance and concluded that nine lines exhibited moderate levels of antibiosis resistance to *M. sacchari*. In a subsequent field study,

Sharma et al. (2014) evaluated 102 sorghum genotypes under natural and artificial infestation identifying seven lines with moderate levels of constitutive resistance to aphid damage.

Investigations using sorghum plant introductions (PI) were significant when greenbug (*Schizaphis graminum* Rondani) shifted hosts from wheat to sorghum (Michels and Burd 2007). Greenbug causes yield losses and lower economic returns due to direct (feeding) and indirect (toxin injection that stops local photosynthesis resulting in chlorosis and predisposing sorghum to charcoal rot) injury, which results in slow plant growth and death. Shortly after shifting from wheat fields to sorghum fields (1969), over 100,000 ha of the crop was destroyed, and millions of hectares had to be sprayed for greenbug. By 1981, it was ranked as the second most damaging insect pest of sorghum in the United States (Michels and Burd 2007). After the first outbreaks, antibiosis and tolerance mechanisms of resistance were identified (Koch et al. 2014), and sorghum hybrids with resistance to biotype “C” greenbug were used as parental lines in commercial sorghum hybrid development (Royer et al. 2015). The research on the development of resistant sorghum hybrids insured the economic viability of sorghum production in the United States (Michels and Burd 2007).

Therefore, the objective of the present work was to characterize levels of resistance to *M. sacchari* in sorghum PIs in the laboratory, greenhouse, and field experiments, and to determine specific host plant resistance traits using the electrical penetration graph (EPG) technique.

5.2 Material and methods

Aphid Colony. The sugarcane aphid colony used in these experiments was founded from a single apterae field collected from sorghum at the Louisiana State Agricultural Center Dean Lee Research Station, Alexandria, LA, in July 2014 by J. A. Davis. This colony, designated LSU-SCA14, was maintained on Pioneer 85G85 (Pioneer Hi-Bred International, Inc., Johnston, IA) planted in 10-cm-dia plastic pots containing sterile potting mix (Sun Gro Horticulture, Elma, MB) and 5 g Osmocote (14-14-14), a slow-release fertilizer (The Scotts Company, Marysville, OH). Plants were grown in a Percival E-36L2 Plant Growth Chambers (Percival Scientific, Perry, IA) held at $25 \pm 0.2^{\circ}\text{C}$, $50 \pm 5\%$ RH and a photoperiod of 14:10 (L:D).

Test Plants. Genotypes with known resistance and susceptibility to greenbug, were requested from the USDA-National Plant Germplasm System (Table 5.1). Seeds of each genotype were planted in plastic pots (11.4 x 15.2 x 15.2 cm) (Model Plastic Nursery Pots Azalea Style, Pöppelmann TEKU®, Claremont, NC) using commercial organic soil for seedlings (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) supplemented with 5 g Osmocote (14-14-14). Pots were maintained in the greenhouse at 22 to 28°C under natural lighting. When the plants reached four to six weeks old, they were used in the experiments.

Table 5.1. Genotypes requested from the USDA-National Plant Germplasm System, their origins and levels of resistance to greenbug (1 = highly resistant and 9 = highly susceptible).

Accession	Origin	Resistance Level
PI220248	Italy, Sicily	3
PI266965	Former Soviet Union	3
PI453951	Ethiopia	9
PI456889	Ethiopia	9
PI457026	Ethiopia	9
PI457174	Ethiopia	9
PI457530	Ethiopia	9
PI457716	Ethiopia	9
PI494893	Zambia	3
PI524770	Zambia	3
PI564163	United States	unknown
PI643515	United States	unknown

Laboratory assays. The study was conducted at 25°C in climate regulated chambers (Model I-41VL, Percival Scientific, Perry, IA) using excised leaves of each genotype following procedures by van Schelt (1994) outlined in van Lenteren (1997) and adapted by Sampaio et al. (2001). Leaf sections of approximately 2 x 3 cm were placed in 30 mL Solo (Dart Container Corporation, Mason, MI) cups filled with 15 ml of a 0.1% agarose (wt/vol) (RM301-500G Agar Powder Extra Pure, HiMedia, Einhausen, Germany). Leaf sections were placed on the surface of the agarose with the abaxial surface upward and replaced every 4 days. This method avoided dehydration of the leaves and prevented aphids from escaping from the leaf sections. A single apterous adult was placed on the leaf section using a moist hair paintbrush (21 x 3 x 2 cm) (Model ARTZ-8009, Arteza, USA) and allowed to reproduce for 24 h. The adult aphid was then removed, leaving only one nymph per leaf section. All single first instars were the cohort for that temperature regimen

for the duration of the experiment; 50 nymphs per cohort replicated in three separate experiments. The cohort was evaluated every 24 hours until death. Development time, juvenile mortality, survivorship, fecundity, and longevity were recorded and measured.

Greenhouse. Genotypes PI220248, PI453951, PI456889, PI457026, PI524770, PI564163 and PI64315 were used to determine genotype suitability to *M. sacchari*. Seeds of each genotype were planted in plastic pots as mentioned before. Pots were maintained in the greenhouse at 22 to 28°C under natural lighting. Each pot contained two plants, with individual plants serving as experimental units.

When the plants reached four to six weeks old, they were used in the experiments. Using a camel hair paintbrush, plants were infested with 10 fourth instar or adults of *M. sacchari*. Infested plants were maintained in 60 × 60 × 60 cm insect cages (MegaView Science Co. Ltd. Taichung, Taiwan). Ten replicates (i.e., individual plants) (n = 10) of each genotype were infested, and the entire experiment was repeated twice (N = 20). Plants were evaluated after seven days for number of individual aphids per plant.

Field trials. The trials were conducted at the Louisiana State University Agricultural Center Ben Hur Research Station (Baton Rouge, LA; 30°22'12.3" North and 91°10'11.6" West) and Louisiana State University Agricultural Center Rice Research Station (Crowley, LA; 31°14'32.5" North and 91°56'06.9" West). Sorghum genotypes PI220248, PI453951, PI456889, PI457026, PI524770, PI564163, and PI64315 were used in the experiment.

Randomized complete block design with three blocks in a factorial treatment structure of 7 (seven) genotypes x 2 (two) localities (2^7) was used (Fig. 1). Because the supply of genotype seeds was limited, the best experimental design was to plant 30 seeds of each genotype in one meter rows. Each block had seven rows, each row corresponding to one genotype, spaced 1 meter between rows (Fig. 1). Genotypes were hand planted on 15 May, 2017 at Ben Hur Research Station and 7 July, 2017 at the Rice Research Station. Sixty and 62 days after planting on Ben Hur Research Station and Rice Research Station, respectively, the plants were collected, placed in paper bags (12 x 7 x 17 inches) and taken to the laboratory for counting of aphids and natural enemies.

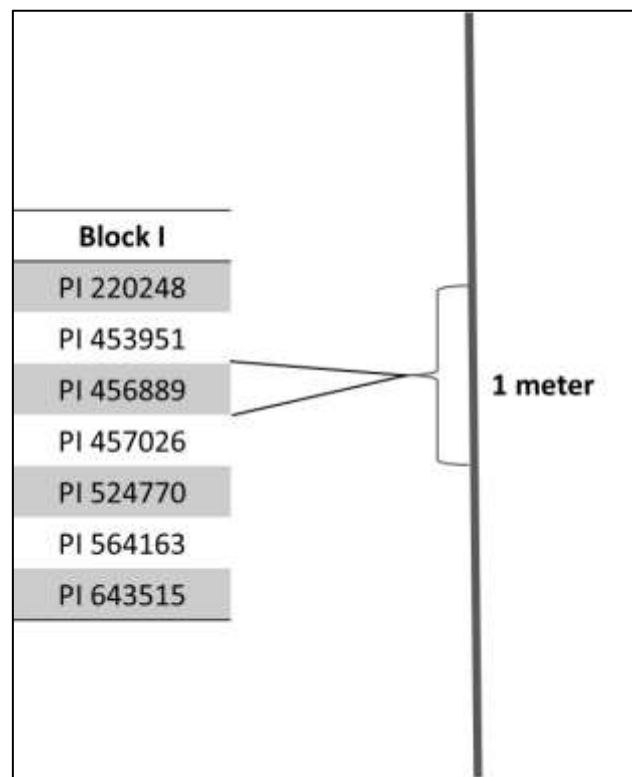


Figure 5.1. Schematic design of field trials showing in detail Block 1.

EPG. EPG experiments were conducted in a Faraday cage using a Giga 8DC EPG amplifier with 1-gigaohm input resistance and an AD conversion rate of 100 Hz (Wageningen Agricultural University, Wageningen, The Netherlands). A DAS-800 Digital Acquisition Card (Keithley Instruments, Inc., Cleveland, OH) digitalized analog signals, which were displayed and recorded using WinDaq/Lite software (DATAQ Instruments, Inc., Akron, OH). A 4-cm gold wire (GoodFellow Metal Ltd., Cambridge, United Kingdom) of diameter 25- μ m was attached to the aphid dorsum with silver conductive paint (Pelco Colloidal Silver no. 16034, Ted Pella, Inc., Redding, CA). The other end of the gold wire was connected by the silver paint to one end of a piece of flattened copper wire peg. After tethering, the flat pegs with wired aphids were connected at the loop end to the monitor input electrodes and held in place over the test plant by metal stands. Two adults were placed on the adaxial surface of the top leaves of one sorghum plant. Then EPG monitoring began.

Feeding behavior was recorded for 4 h, based on other studies indicating that this time frame was sufficient for *M. sacchari* to penetrate the leaf tissue to the sieve elements (Akbar et al. 2014, Fartek et al. 2012). There were two plants per recording, therefore four aphids per recording. Twelve aphids were recorded on each genotype with a total of 84 aphids and 336 h of recording. The beginning and the end of each EPG waveform in all EPG recordings were marked manually and subsequently, the 17 EPG parameters representing aphid probing and feeding behaviors were calculated.

Measured parameters included the start and end of each probe, number of probes, time to start the first probe, time to reach sieve elements phase (from first probe), start and end of each individual phloem phase and xylem phase, numbers of cell punctures (potential drops) to

reach sieve element phase, total numbers of potential drops during the time of the recording, number of events for each phase (i.e., number of E1, E2, E2>10min, xylem). Based on these readings, we calculated the total penetration time (sum of all probing time within a 4-h period), non-probe time, total time in pathway phase (time probing excluded phloem and xylem phases), mean probe duration (total time probing/number of probes), total duration of xylem phase (sum of all time in xylem phase), sum of time spent in each individual phloem phase (E1, E2), and cell puncture frequency per minute (total pds/time probing). Additionally, the proportion of individuals reaching phloem and xylem phase were also calculated.

Statistical Analysis. The biological variables (longevity, development time, nymphs per female, reproductive period, nymphs per female per day, and juvenile mortality) on no-choice laboratory tests and the number of aphids per plant on greenhouse tests were analyzed using a complete randomized design. PROC MIXED procedures were used for all datasets with sorghum genotypes as fixed effects and the trials considered random effects. Tukey-HSD analysis at 0.05% of significance allowed us to compare the least square means and determine whether genotypes effects were significant for each variable.

Melanaphis sacchari age-specific survival (l_x) and fecundity (m_x) were calculated for each sorghum genotype. Net reproductive rate, R_o , defined as the product of age-specific survival and age-specific fecundity, was calculated using the formula $R_o = \sum l_x m_x$, where l_x is the proportion of females alive on a given day, and m_x is the mean number of female births on that day. The intrinsic rate of increase, r_m , ($\sum e^{-r_m} l_x m_x = 1$), finite rate of increase ($\lambda_F = e^{r_m}$), mean generation time [$T_G = \ln R_o / r_m$], and doubling time (DT = $\ln(2) / r_m$) of a generation were

estimated according to Birch (1948). Jackknifing procedure was used to estimate r_m standard error. This procedure is based on recombining the original data and calculating pseudo-values of r_m for each recombination of the original data and estimating the mean value and standard error of r_m from the resulting frequency distribution of pseudo-values in accordance with Meyers et al. (1986). Mean generation time, net reproductive rate, doubling time, and finite rate of increase were analyzed by one-way ANOVA in PROC MIXED with host plants as independent fixed factors. Treatment means were separated by the Tukey-HSD test ($\alpha = 0.05$) (SAS 9.4, Institute Inc., Cary, NC, 2013).

In field trials, the differences in aphid and natural enemies numbers recorded on each genotype and location were analyzed using PROC MIXED on SAS 9.4 by using the analysis of variance for randomized complete block. For purposes of analysis, we assigned block as a random effect and local and genotype as fixed effects.

The effect of sorghum genotypes on the proportion of individuals showing any specific EPG parameter was tested by a generalized linear model fitted in an exact logistic function since this method is more appropriate for small-sample clustered binary data (Mehta and Patel 1995). The PROC LOGISTIC procedure on SAS 9.4 was used for this finality.

Total time *M. sacchari* spent in a certain waveform and the number of occurrences of a particular event during 4 h recording period were tested using one-way ANOVA in PROC GLM. Levenes's test for homogeneity of variances and Shapiro-Wilk to test the normality of data were performed previously to ANOVA. In case of non-normality of the errors or inequality of variances (Total cell punctures, Total duration of sieve element salivation, Total duration of the pathway phase, Table 4, #10, #27 and #15) Welch's' ANOVA was applied.

All the statistical analysis were performed on SAS 9.4 (SAS 9.4, Institute Inc., Cary, NC, 2013), and SigmaPlot (SigmaPlot 14.0, Systat Software Inc., San Jose, CA, USA, 2018) was used to plot age-specific survival graphs, greenhouse and field data.

5.3 Results

Life table studies. Sorghum genotype had a significant effect on the survival of the *M. sacchari* (Table 5.2). Genotypes PI524770 and PI643515 showed the greatest immature mortality, with more than 36.0 and 44.7% of the cohort dying in the juvenile phase, respectively. Nymphs on PI457174 had only 5.3% mortality and on genotypes PI220248, PI453951, PI456889, PI457026, and PI4577716, about 10% of the cohort did not survive until adulthood (Table 5.2).

Melanaphis sacchari development time was significantly affected by the sorghum genotype (Table 5.2). On genotypes PI524770 and PI457026, *M. sacchari* took the longest time to reach adulthood, and on genotypes PI453951 and PI456889 *M. sacchari* reached adult the fastest (Table 5.2).

Adult *M. sacchari* readily larviposited, and the time that aphids remained reproductively active had significant variance according to the sorghum genotype (Table 5.2). *Melanaphis sacchari* reproduced actively for the shortest period on PI524770, PI564163, and PI643515, but on PI220248 the mean reproductive time of an individual was the longest (12.5 days).

Table 5.2. Biological variables of *M. sacchari* confined on different sorghum PI genotypes. Means followed by different letters on the column are different by Tukey-HSD test at 5% of significance.

Genotype	n	Immature mortality (%)	Development time (d)	Reproductive period (d)	Nymphs per female	Longevity (d)
PI220248	150	10.0 ± 0.0 A	4.8 ± 0.1 AB	12.5 ± 0.6 E	49.0 ± 2.3 CD	19.3 ± 0.8 D
PI266965	150	18.7 ± 0.0 AB	4.7 ± 0.1 AB	7.5 ± 0.5 B	40.5 ± 2.1 BC	13.6 ± 0.6 B
PI453951	150	10.0 ± 0.0 A	4.5 ± 0.1 A	11.1 ± 0.4 DE	60.8 ± 1.9 E	16.4 ± 0.4 C
PI456889	150	10.7 ± 0.0 A	4.5 ± 0.1 A	9.9 ± 0.4 CD	61.9 ± 2.3 E	15.1 ± 0.5 BC
PI457026	150	10.0 ± 0.0 A	5.4 ± 0.1 E	8.1 ± 0.4 BC	39.2 ± 2.3 BC	13.8 ± 0.5 BC
PI457174	150	5.3 ± 0.0 A	5.0 ± 0.1 BCD	9.5 ± 0.4 BCD	52.3 ± 2.1 DE	16.1 ± 0.6 BC
PI457530	150	15.3 ± 0.0 AB	5.2 ± 0.1 DE	8.5 ± 0.5 BC	36.5 ± 2.0 B	14.2 ± 0.6 BC
PI457716	150	10 ± 0.0 A	5.3 ± 0.1 DE	9.4 ± 0.5 BCD	45.8 ± 2.2 BCD	15.0 ± 0.6 BC
PI494893	150	14.0 ± 0.1 A	5.0 ± 0.1 BCD	9.5 ± 0.5 BCD	43.8 ± 2.6 BCD	15.7 ± 0.6 BC
PI524770	150	44.7 ± 0.1 C	5.4 ± 0.1 E	4.9 ± 0.5 A	19.5 ± 2.0 A	9.8 ± 0.6 A
PI564163	150	25.0 ± 0.0 ABC	5.1 ± 0.1 CDE	3.2 ± 0.4 A	11.8 ± 1.6 A	9.3 ± 0.5 A
PI643515	150	36.0 ± 0.1 BC	4.9 ± 0.1 BC	3.2 ± 0.3 A	14.5 ± 1.6 A	7.4 ± 0.4 A
<i>F</i>		7.554	23.281	38.398	55.956	34.35
<i>P</i>		<0.001	<0.001	<0.001	<0.001	<0.001

During the reproductive period, the mean number of nymphs per female ranged from 11.8 to 61.9, but some individuals produced as many as 112 nymphs. Genotype significantly influenced the number of nymphs produced by *M. sacchari*, with aphids on genotypes PI453951 and PI456889 producing about 25% more nymphs than on PI524770, PI564163, and PI643515, (Table 5.2). *Melanaphis sacchari* longevity ranged from 7.4 on PI643515 to 19.3 days on PI220248, and the difference between the genotypes was significant (Table 5.2).

Table 5.3. Populations parameters of *M. sacchari* confined on different sorghum PI genotypes.

Genotype	Intrinsic rate of increase			Net reproductive rate	Mean generation time (Days)	Doubling Time (Days)	Finite rate of increase
	n	r_m	SE				
PI220248	150	0.415	± 0.007	47.5 ± 0.01 D	9.3 ± 0.001 F	1.7 ± 0.0002 H	1.5 ± 0.0001 E
PI266965	150	0.429	± 0.008	38.8 ± 0.01 G	8.4 ± 0.001 H	1.6 ± 0.0002 I	1.5 ± 0.0001 D
PI453951	150	0.471	± 0.025	53.2 ± 0.03 A	8.4 ± 0.002 I	1.5 ± 0.0006 K	1.6 ± 0.0003 B
PI456889	150	0.478	± 0.053	48.0 ± 0.05 C	8.1 ± 0.003 K	1.5 ± 0.0012 L	1.6 ± 0.0006 A
PI457026	150	0.372	± 0.007	37.5 ± 0.01 H	9.7 ± 0.001 C	1.9 ± 0.0002 E	1.5 ± 0.0006 H
PI457174	150	0.454	± 0.011	50.0 ± 0.01 B	8.6 ± 0.004 G	1.5 ± 0.0003 J	1.6 ± 0.0001 C
PI457530	150	0.356	± 0.016	32.1 ± 0.02 I	9.7 ± 0.002 D	1.9 ± 0.0006 D	1.4 ± 0.0002 I
PI457716	150	0.380	± 0.015	45.6 ± 0.01 E	10.1 ± 0.003 A	1.8 ± 0.0005 F	1.5 ± 0.0001 G
PI494893	150	0.390	± 0.007	42.7 ± 0.01 F	9.6 ± 0.001 E	1.8 ± 0.0002 G	1.5 ± 0.0001 F
PI524770	150	0.294	± 0.011	18.2 ± 0.01 J	9.9 ± 0.001 B	2.3 ± 0.0006 B	1.3 ± 0.0001 K
PI564163	150	0.273	± 0.016	9.9 ± 0.02 L	8.4 ± 0.002 J	2.5 ± 0.0012 C	1.3 ± 0.0002 L
PI643515	150	0.321	± 0.018	12.5 ± 0.02 K	7.8 ± 0.003 L	2.1 ± 0.0008 A	1.4 ± 0.0001 J
<i>F</i>				89858.178	130903.080	271299.210	201425.787
<i>p</i>				< 0.0001	< 0.0001	< 0.0001	< 0.0001

r_m = Intrinsic rate of increase, SE = standart error

Melanaphis sacchari population parameters were calculated for each sorghum genotype (Table 5.3). The greatest r_m observed was on PI456889, 1.8-times higher than the intrinsic rate of increase of *M. sacchari* on PI564163 (Table 5.3). Intermediate r_m were observed on PI457026, PI457716, PI457530, PI494893, PI643515 (Table 5.3).

Melanaphis sacchari mean generation time, T_G , was the shortest on PI643515 (7.8 days), which was 20% longer than on PI457716 (the longest T_G) (Table 5.3). Net reproductive rate (R_0) estimates how many times a population can grow from one generation to another. *Melanaphis sacchari* cohorts developing on PI453951 had the highest R_0 of 53.2, 5X greater than the cohort reared on PI564163, which had the lowest net reproductive rate (Table 5.3). The plant genotype on which *M. sacchari* developed influenced doubling time (DT) and finite rate of increase (λ) (Table 5.3). *Melanaphis sacchari* populations reared on PI524770, PI564163, and PI643515 needed more than two days to double the population while on the other genotypes, populations could double in less than two days. Finite rate of increase had low variability, ranging from 1.3 to 1.6 (Table 5.3).

In Fig. 5.2, age-specific survivorship (l_x) for *M. sacchari* reared on the 12 sorghum genotypes is shown. When *M. sacchari* developed on genotype PI220248, we observed the longest age-specific survival with aphids living up to 40 days. Individuals on genotype PI453951 also had a slow death rate, with 50% survival until day 18. Genotype PI457174 also provided a remarkable survival of *M. sacchari*, with individuals alive until day 36. Individuals on genotypes PI643515, PI 524770 and PI564163 showed faster death rates than on the other genotypes. On genotype PI643515, individuals reached 50% mortality on day 8 and on PI524770 and PI564163, this rate was observed on day 9. (Fig. 5.2).

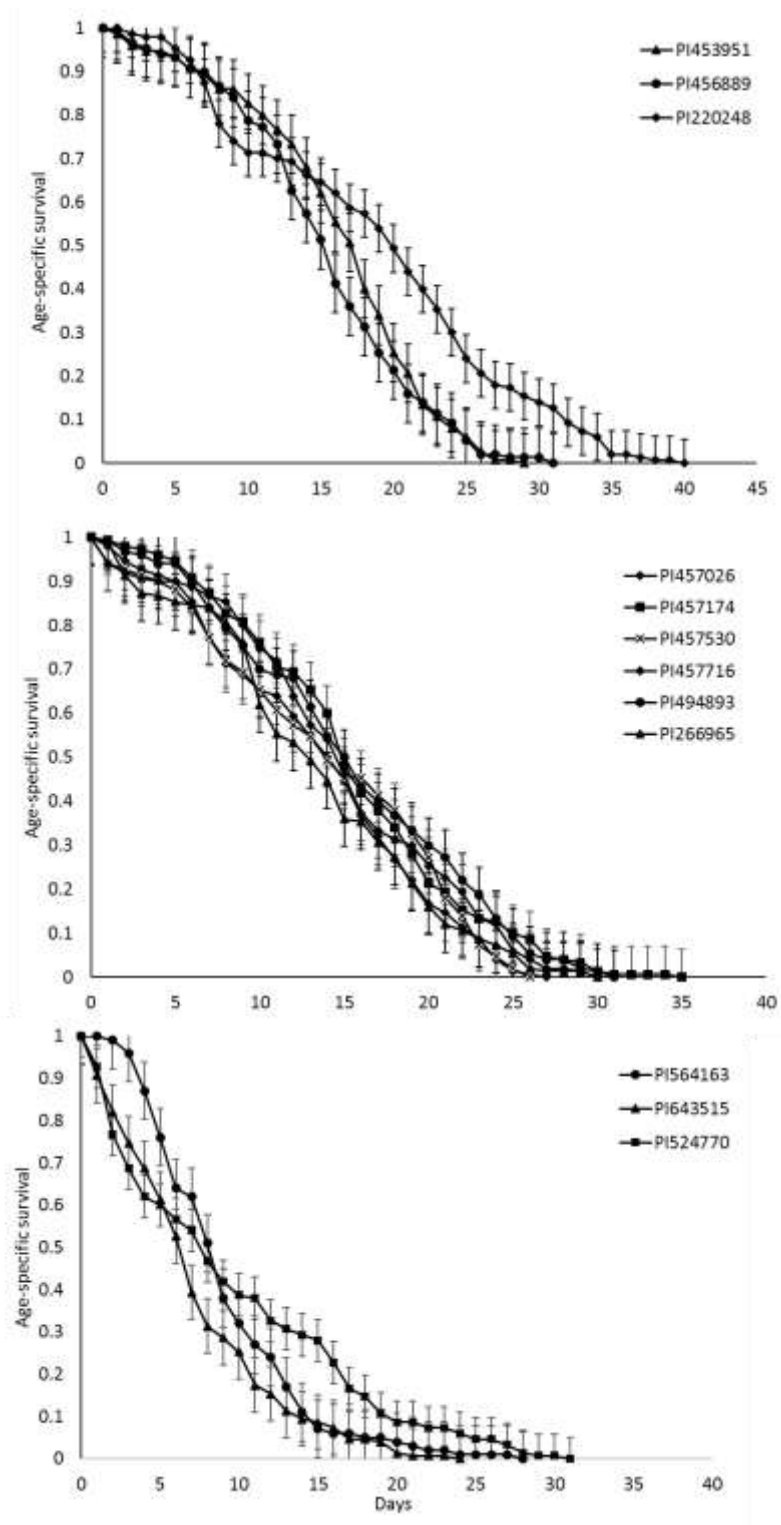


Figure 5.2. Age-specific survival of *M. sacchari* reared on 12 different sorghum plant introduction (PI) genotypes.

Greenhouse. In greenhouse tests, PI220248, PI453951, PI456889, PI457026, PI524770, PI564163 and PI643515 were studied. Genotype affected the number of apterous aphids per plant after a week ($F = 11.117$, $p > 0.001$), but had no effect on number of alatae per plant ($F = 1.577$, $p = 0.1582$) (Fig. 5.3).

Genotypes PI220248, PI457026, and PI456889 had the highest number of aphids per plant. After a week on genotypes PI220248, PI457026, and PI456889, the 10 initial aphids grew their population to 231.8, 198.9, and 221.0 aphids per plant. Genotypes PI453951 and PI564163 showed intermediate rates of aphid multiplication with 191 and 109 apterous per plant, respectively. Less than a hundred aphids per plant were counted on genotypes PI524770 and PI643515, where 91.9 and 72.4 aphids per plant were observed.

Alatae numbers were very low on genotypes PI524770 and PI453951, in which the mean number per plant was lower than one. On genotypes PI643515, PI564163, PI456889, PI457026, the mean alatae per plant ranged from 1.7 to 3.6, while on PI220248, 5.5 alatae per plant was the mean observed.

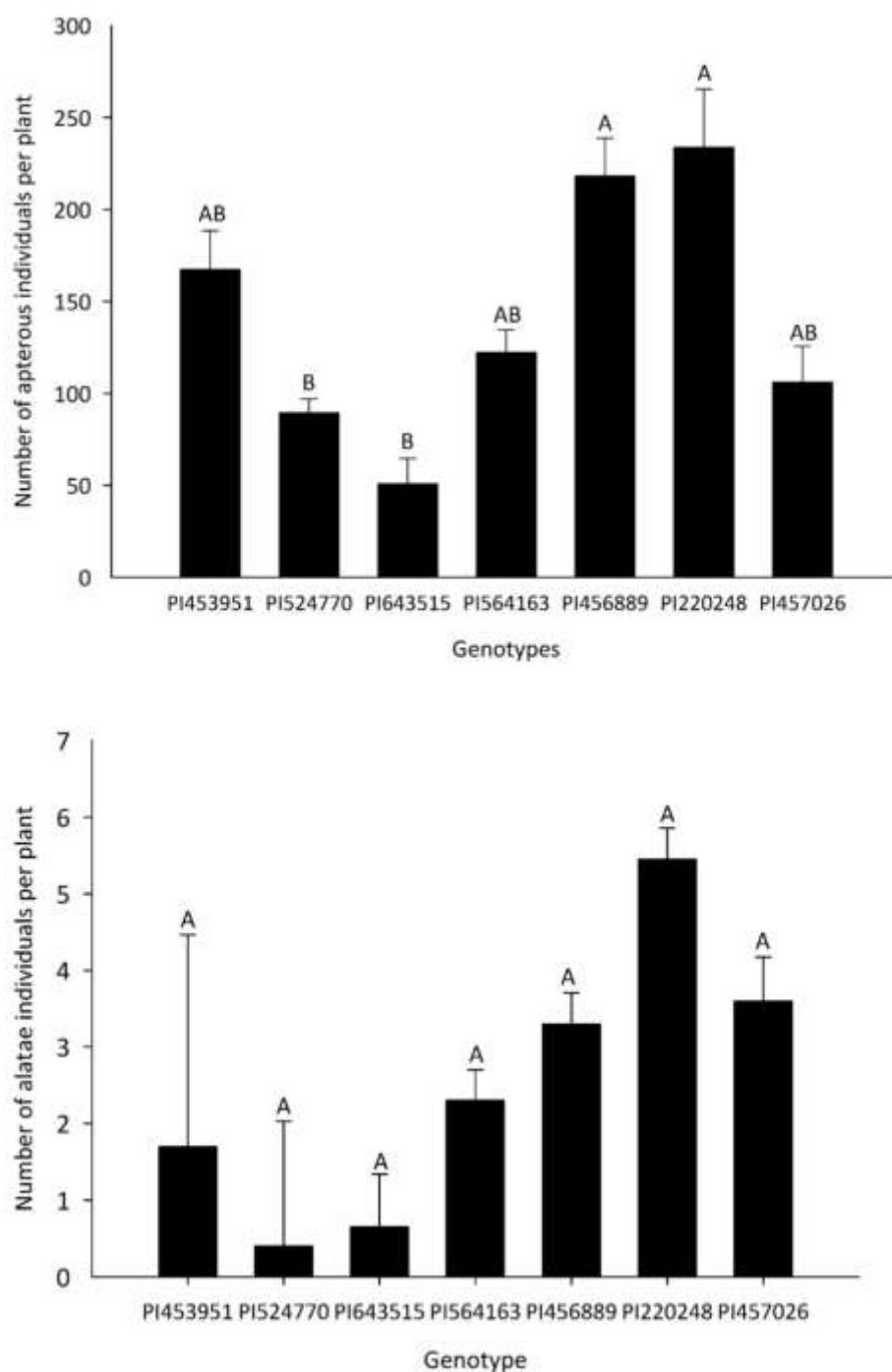


Figure 5.3. Number of apterous and alatae *M. sacchari* individuals on sorghum PIs genotypes seven days after infestation on greenhouse. Different letters on top of the bars indicate significant difference for number of apterous aphids per plant using Tukey-HDS ($\alpha = 0.05$).

Field trial. The interaction between localities (Ben Hur Research Station or Rice Research Station) and genotype was not significant for total number of aphids ($F = -1.099$, $p = 1.0000$) and for number of natural enemies ($F = 0.323$, $p = 0.9244$).

The total number of aphids per plant was significantly affected by the location in which the genotypes were planted ($F = 19.184$, $p < 0.0001$), and by the genotype considered ($F = 6.872$, $p < 0.0001$) (Fig. 5.4). At the Rice Research Station, the total number of aphids per plant was 8-fold greater than at the Ben Hur Research Station, regardless of the genotype. Even having a greater number of aphid per plant, the maximum amount of aphid observed on Rice Research Station was 518 aphids on one plant (Fig. 5.4).

On genotype PI220248, 69.5 aphids per plant were observed, the greatest counting per genotypes screened. An average of 20.9 and 6.9 aphids per plant were observed on PI 456889 and PI 453951, and on the remaining genotypes, the mean was less than one aphid per plant. On genotype PI564163 no aphids or natural enemies were observed, and on genotypes PI524770, PI643515, PI457026, the average was less than one aphid per plant (Fig. 5.5).

The natural enemies observed on these genotypes were mainly ladybugs (Coleoptera: Coccinellidae), syrphid larvae (Diptera: Syrphidae), earwigs (Dermaptera: Forficullidae), minute pirate bugs (Hemiptera: Anthocoridae), and spiders. The number of natural enemies did not vary with genotype ($F = 0.743$, $p = 0.6359$) (Fig. 5) but only with the location ($F = 6.421$, $p = 0.0122$) (Fig. 5.5).

EPG. *Melanaphis sacchari* spent from 11 to 59% of the 4-h experimental period non-probing on the tested genotypes (Table 5.4, #2), and cultivar differences were detected on both non-probing time ($F = 3.488$, $p = 0.0063$) and on total penetrating time ($F = 5.80$, $p > 0.0001$) (Table 5.4, #3). Total probing time was 3.6-times longer on PI453951 than on PI220248. However mean probe duration did not differ among genotypes ($F = 2.08$, $p < 0.0711$) (Table 5.4, #5).

Most probing time was in the pathway phase, with cultivar influencing this parameter ($F = 4.73$, $p = 0.0012$) (Table 5.4, #11). On genotypes PI564163 and PI643515, *M. sacchari* spent 68.0 and 75.4% of the 4 h on pathway phase while only 33 to 38% on genotypes PI220248, PI453951, and PI456889. In the sieve element phase, the total time spent in E1 averaged 2.4 min on PI453951, PI456889, and PI457026. These E1 times were significantly different from the 19 min spent in PI564163 (Table 5.4, #12). Meanwhile, the time in E2 was 4.6-times greater on PI220248 than on PI643515 ($F = 3.277$, $p = 0.0113$) (Table 4, #13).

Melanaphis sacchari took an average of 31 min to start probing regardless of cultivar ($F = 1.69$, $p = 0.1409$) (Table 5.4, #1), and the number of probes was 4.2-times greater on PI643515 than on PI220248 ($F = 2.95$, $p = 0.0148$) (Table 5.4, #4). The total number of cell punctures during the 4 h recording ($F = 1.41$, $p = 0.2323$) (Table 5.4, #16) and cell puncture frequency per minute ($F = 0.70$, $p = 0.6532$) (Table 5.4, #17) were not affected by genotype. Proportions of aphids reaching phloem at least once were $> 50\%$ among the seven genotypes ($\chi^2 = 14.7377$, $p = 0.0224$) (Table 5.4, #6), with all the individuals starting phloem phase on PI453951, which was significantly greater than the proportion of individuals reaching phloem on PI220248, PI524770 and PI643515.

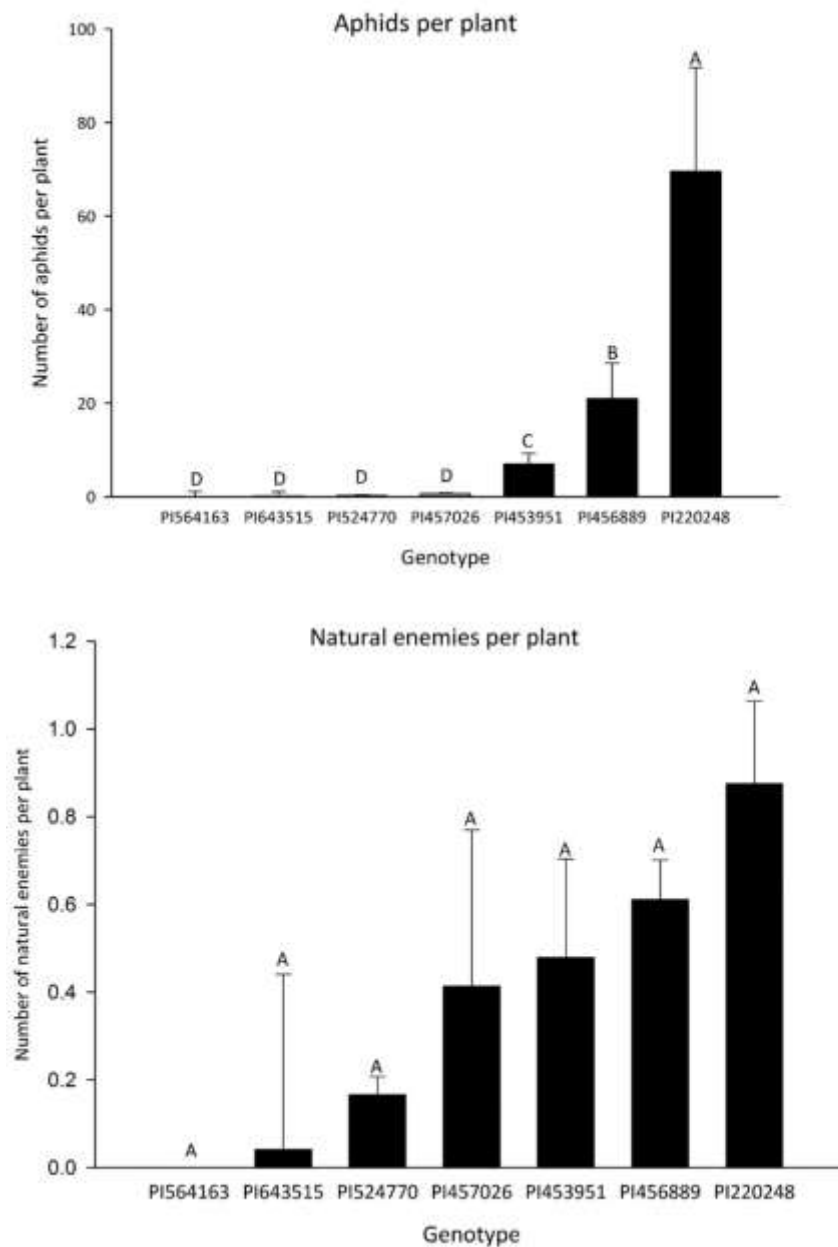


Figure 5.4. Mean number of individuals *M. sacchari* and natural enemies per plant at Ben Hur Research Station and Rice Research Station in 2017. Different letters on the bars indicate difference at 5% using Tukey-HSD test.

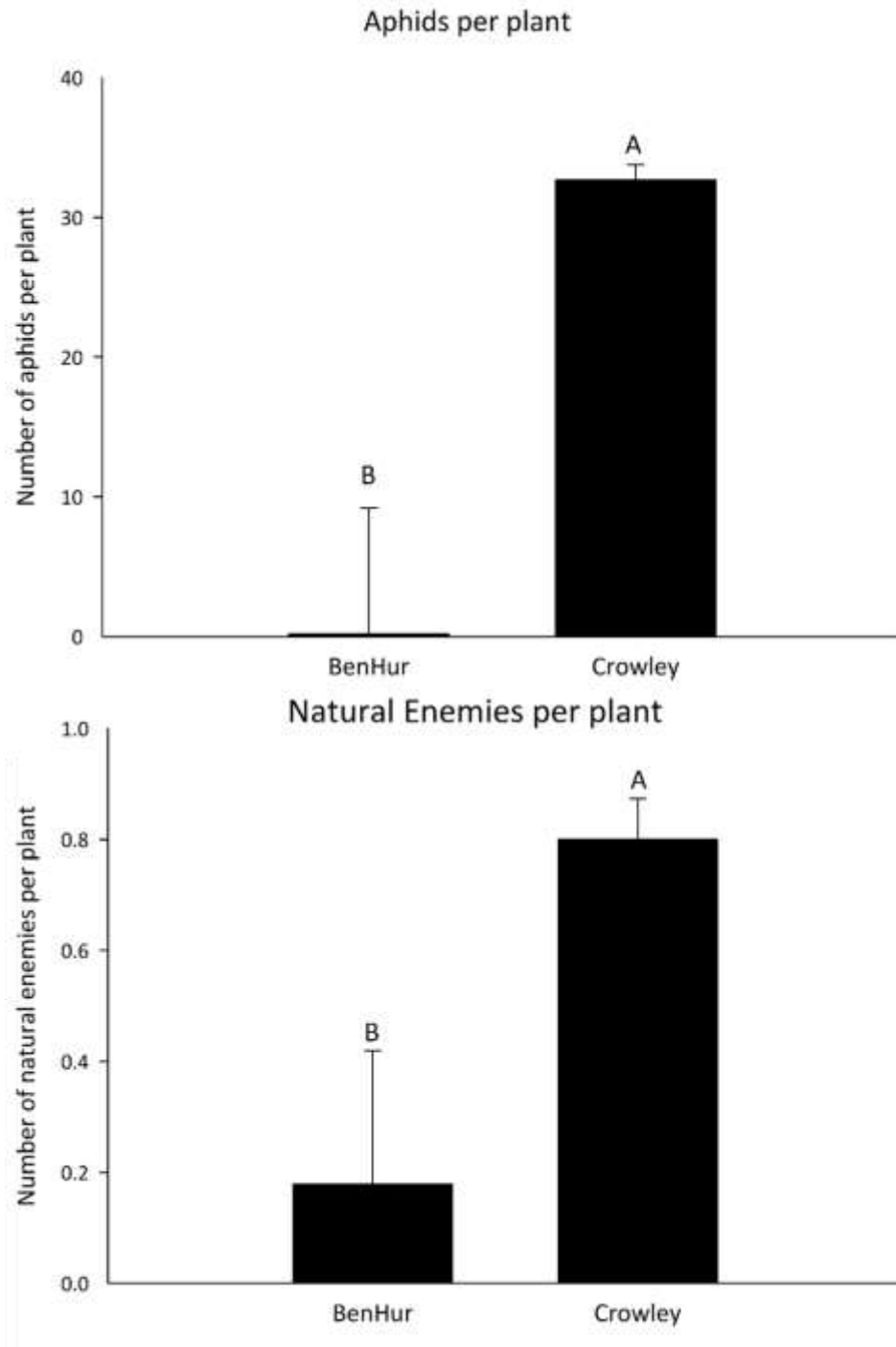


Figure 5.5. Mean number of individuals *M. sacchari* and natural enemies per plant in 2017. Different letters on the bars indicate difference at 5% using Tukey-HSD test.

The time required for commencing contact with phloem vessels after onset of the first probe was unaffected by genotype ($F = 2.10$, $p = 0.0770$) (Table 5.4, #7). The numbers of phloem salivation events ($F = 0.83$, $p = 0.5569$) (Table 5.4, #8), feeding occurrences ($F = 0.27$, $p = 0.9488$) (Table 5.4, #9) and feeding events longer than 10 minutes ($F = 2.31$, $p = 0.0540$) (Table 5.4, #10) were also unaffected by genotype, but the proportion of individuals showing xylem phase was affected by genotype ($X^2 = 17.8053$, $p = 0.0067$) (Table 5.4, #14). Aphids on genotype PI220248 never started xylem phase and only one aphid reached xylem phase on PI456889 and PI643515, therefore these genotypes were excluded of the analysis. Most of the individuals ingested xylem on genotype PI457026 (Table 5.4, #14). However the total duration of xylem ingestion was not affected by genotype ($F = 0.30$, $p = 0.9013$) (Table 5.4, #15).

Table 5.4: Feeding behavior parameters (mean \pm SE) of *M. sacchari* during a 4-h period on seven different sorghum genotypes.

		Sorghum Genotype						
EPG parameter		PI220248	PI453951	PI456889	PI457026	PI524770	PI564163	PI643515
1.	Time from start of experiment to probe	3882.7 \pm 1658.9 A	957.3 \pm 616.3 A	1984.1 \pm 611.6 A	436.5 \pm 128.8 A	2391.9 \pm 1624.4 A	396.1 \pm 79.8 A	3075.3 \pm 1883.2 A
2.	Non-probing time (s)	8543.2 \pm 3273.7 AB	2633.4 \pm 766.7 A	4123.2 \pm 874.9 AB	4806.6 \pm 1787.0 AB	7526.9 \pm 2511.8 B	1607.3 \pm 243.5 A	7202.1 \pm 2529.9 AB
3.	Total penetration time	3040.6 \pm 1911.0 B	11944.0 \pm 791.3 A	10542.8 \pm 874.9 A	9783.4 \pm 1781.7 A	7540.9 \pm 2128.8 AB	13058.7 \pm 243.5 A	7463.9 \pm 2529.9 AB
4.	Number of probes	2.8 \pm 0.8 A	6.0 \pm 1.1 AB	6.2 \pm 1.1 AB	5.9 \pm 1.9 AB	9.8 \pm 4.0 AB	9.9 \pm 0.8 AB	12.0 \pm 3.3 B
5.	Individual probing duration	615.7 \pm 398.0 A	3002.0 \pm 565.3 A	3057.0 \pm 1102.7 A	3155.0 \pm 1376.0 A	873.1 \pm 337.9 A	1418.6 \pm 138.1 A	771.3 \pm 248.5 A

(Table cont`d)

Table 5.4: Continued. Feeding behavior parameters (mean \pm SE) of *M. sacchari* during a 4-h period on seven different sorghum genotypes.

		Sorghum Genotype						
EPG parameter		PI220248	PI453951	PI456889	PI457026	PI524770	PI564163	PI643515
6.	Proportion of individuals showing SE phase	0.56 B	1.0 A	0.92 AB	0.71 AB	0.50 B	0.90 AB	0.57 B
7.	Time from first probe to first SE phase	3447.4 \pm 66.9 A	3585.8 \pm 1114.6 A	2747.7 \pm 769.6 A	5102.3 \pm 1718.1 A	7442.9 \pm 2007.1 A	7309.2 \pm 1318.2 A	5522.3 \pm 1244.1 A
8.	Number of SE salivation events	10.5 \pm 9.5 A	5.0 \pm 1.3 A	4.7 \pm 1.0 A	4.0 \pm 0.7 AB	3.3 \pm 1.9 A	4.1 \pm 1.0 A	4.3 \pm 1.6 A
9.	Number of SE feeding events	2.5 \pm 1.5 A	2.0 \pm 0.4 A	2.5 \pm 0.4 A	2.8 \pm 0.6 AB	2.3 \pm 1.3 A	2.3 \pm 0.3 A	2.0 \pm 0.4 A
10.	Number of SE sustained feeding events	1.0 \pm 0.0 A	1.6 \pm 0.3 A	1.7 \pm 0.3 A	1.2 \pm 0.4 A	0.3 \pm 0.3 A	1.1 \pm 0.3 A	0.3 \pm 0.3 A
11.	Total duration of pathway phase	4773.5 \pm 2486.9 B	5519.1 \pm 798.0 B	5077.6 \pm 527.1 B	6441.9 \pm 1846.0 AB	5849.6 \pm 1590.7 AB	9791.8 \pm 624.1 A	10870.0 \pm 1271.6 A

(Table cont'd)

Table 5.4: Continued. Feeding behavior parameters (mean \pm SE) of *M. sacchari* during a 4-h period on seven different sorghum genotypes.

EPG parameter	Sorghum Genotype						
	PI220248	PI453951	PI456889	PI457026	PI524770	PI564163	PI643515
12. Total duration of SE salivation	518.2 \pm 474.1 AB	136.0 \pm 36.9 B	165.6 \pm 31.9 B	136.0 \pm 49.3 B	662.1 \pm 371.9 AB	1192.1 \pm 461.3 A	297.8 \pm 246.5 AB
13. Total duration of SE feeding	7429.0 \pm 2128.0 B	6107.5 \pm 983.5 AB	6288.4 \pm 997.7 AB	3415.3 \pm 1645.5 AB	1290.7 \pm 669.1 AB	2350.1 \pm 811.6 AB	1599.2 \pm 1216.2 A
14. Proportion of individuals showing xylem* ingestion	*	0.17 AB	0.08 B	0.71 A	0.50 A	0.40 A	0.14 AB
15. Total duration of xylem ingestions	*	1156.2 \pm 1124.8 A	*	994.6 \pm 329.7 A	1828.7 \pm 1230.3 A	1839.8 \pm 1023.2 A	*
16. Total cell punctures	53.7 \pm 48.3 A	84.6 \pm 11.2 A	78.0 \pm 11.3 A	94.1 \pm 25.3 A	113.0 \pm 17.1 A	133.4 \pm 16.2 A	96.6 \pm 40.0 A
17. Cell puncture frequency per minute	0.4 \pm 0.4 A	0.5 \pm 0.1 A	0.4 \pm 0.1 A	0.6 \pm 0.1 A	0.7 \pm 0.1 A	0.6 \pm 0.1 A	1.2 \pm 0.6 A

*Treatment excluded from the analysis to avoid infinite estimation of likelihood, see Materials and Methods section.

5.4 Discussion

Life table analysis. The laboratory screening categorized 12 sorghum germplasms into three groups. The first group, composed of two Ethiopian lines, PI453951 and PI456889, and one Italian line PI 220248, were highly suitable for *M. sacchari* population growth. From this first group, PI453951 and PI 456889 are susceptible to greenbug. Genotype PI220248 was consistently antibiotic to greenbug (Schuster and Starks 1973, Dixon et al. 1990). On these genotypes, *M. sacchari* showed the highest intrinsic rates of increase, greatest reproduction, shortest development time, and most extended reproductive period and longevity.

The second group, composed of four Ethiopian genotypes; PI457026, PI457174, PI457530 and PI457716, one Zambian genotype, PI494893; and one from the former Soviet Union, PI266965, was intermediate in its suitability as *M. sacchari* hosts. Levels of resistance in this group ranged from highly susceptible to resistant to greenbug (see Table 1, Material and Methods). Genotypes in this group varied in their effect on *M. sacchari* biology, with some genotypes resulting in inhibition of reproductive parameters, but at the same time shortening development time and increasing longevity and reproduction.

The third group was composed of the remainder of lines in the trial. It included two United States materials, PI564163 and PI643515, and one Zambian, PI524770. These genotypes inhibited reproductive fitness of *M. sacchari* by increased mortality and development time, and shortened longevity. Levels of resistance for greenbug are unknown for genotypes PI564163 and PI643515 while PI524770 is resistant to greenbug.

Effects of genotypes on *M. sacchari* life table parameters were not consistent with greenbug resistance. The specificity of expression of resistance towards an aphid species has been observed before (Robinson 1993, Webster et al. 1987, Rodriguez-Saona et al. 2010, Pitino and Hogenhout 2013, Rodriguez et al. 2017). For instance, resistance to *Diuraphis noxia* in wheat and barley is particularly specific and does not affect other cereal aphid species (Robinson 1993, Webster et al. 1987). Plant-aphid interactions are so specific that variations in the same plant-aphid species system can occur. For instance, Bayoumy et al. (2016) found that *M. sacchari* developed the fastest in the susceptible hybrid (P8500, Pioneer), but Michaud et al. (2017) observed that P8500 delayed *M. sacchari* development. One genotype in the second group, PI457026, two genotypes in the first group (PI453951 and PI220248), and PI524770, PI564163 and PI643515 from the third group were selected for further evaluation in the greenhouse, field tests, and EPG analysis.

Greenhouse. Greenhouse studies using whole plants can be used to monitor multiple individuals with free access to all the plant and access the plant-insect interaction in a less controlled environment (Markkula and Rautapaa 1963, Teetes et al. 1995, Fartek et al. 2012, Armstrong et al. 2015, Sharma et al. 2013). Genotypes PI524770 and PI643515, *M. sacchari* populations reached 70 to 90 aphids per plant, confirming the negative impacts on confined assays. Dixon et al. (1990) observed a count of 25 greenbug individuals on genotype PI220248, which was lower than the present observation of 231.7 *M. sacchari* individuals per plant. Therefore in the present study, whole plant assays confirmed the specificity of resistance to aphids on sorghum plants.

No-choice greenhouse and laboratory studies showed that some genotypes negatively affected *M. sacchari* fitness. The probable causes of fitness reduction on specific genotypes are many. External causes such as physical and morphological differences among the genotypes and internal causes such as toxins and nutritional factors may be altering *M. sacchari* fitness on each genotype (Storer et al. 1996, Powell et al. 1999, Sun et al. 2017). Specific plant compounds, such as hydroxamic acids (Argandona et al. 1983, Givovich and Niemeyer 1995, Fuentes-Contreras and Niemeyer 1998, Hansen 2006), phenols and flavonoids (Leszczynski et al. 1995, Dreyer and Jones 1981), proteins inhibitors (Rahbe et al. 2003, Ribeiro et al. 2006, Losvik et al. 2017), tannins (Dreyer et al. 1981) or the results of different plant signaling defense pathways (Zust and Agrawal 2016, Donovan et al. 2013, De Vos et al. 2009, Coppola et al. 2013) can decrease aphid fitness due to deterrence or toxicity.

Differences in nutrient levels of the genotypes in the present study may also explain the impacts of different genotypes on *M. sacchari* life table parameters. Since aphids feed on a limited diet as plant sap, dietary requirements, especially nitrogen may be a base of plant resistance (Mittler 1967, Srivastava and Auclair 1975, Prosser et al. 1992, Ponder et al. 2000). Ponder et al. (2000) showed that nitrogen deficient barley explains the lowered performance of *R. padi*. Akbar et al. (2014) investigating *M. sacchari* feeding behavior on sugarcane cultivars HoCP 91-555 (resistant), LCP 85-384 (moderately resistant), and L 97-128 (susceptible) observed that cultivar effects were not detected for levels of total phenolics, available carbohydrates, and water potential, but two free essential amino acids, histidine, and arginine, were absent from phloem sap in HoCP 91-555. Aphids feeding time was lower on this resistant cultivar than on the susceptible one (Akbar et al. 2014).

Field trials. In the field trials conducted at two locations with 7 genotypes, Rice Research Station always had the greater number of aphids and consequently greater number of natural enemies. Genotype PI220248 showed the greatest aphid abundance while on genotypes PI564163, PI643515, PI 524770 and PI453951, the number of aphids per plant was low. However, even though PI220248 had up to 600 aphids per plant, this is much lower than the usually observed field infestations but higher than the established action thresholds. Brewer et al. (2017) recorded counts up to 900 aphids per sorghum leaf. Szczepaniec (2018) documented sums exceeding 500 aphids per leaf.

In the present study, Coccinellidae, Forficullidae, and Syphidae were the natural enemies found in both locations on all the genotypes, corroborating with Colares et al. (2015) and Bowling et al. (2016) observations. The different levels of resistance to *M. sacchari* on each genotype did not affect natural enemy abundance. The suppression of aphid populations by host plant resistance may negatively affect biological control (van Emden 1978, Du Li et al. 2004). However, even with the low aphid density on specific genotypes, the natural enemy abundance was the same across genotypes. Therefore, coupling biological control with host plant resistance appears to be a viable option for integrated pest management of sugarcane aphid in sorghum. This study was not designed to test this and further research is necessary to quantify the impact of natural enemies on aphid suppression in resistant sorghum.

The field is a dynamic environment requiring the consideration of extrinsic factors and more complex interactions, including biotic and abiotic factors. For instance, the incidence of heavy rainfall corresponded to declines in greenbug populations on grain sorghum in Central Missouri (Hamilton et al. 1982). During the field trials, the precipitation on Ben Hur Research

Station was 470 mm with rainfall on 53% of the 60 days that the sorghum genotypes were in the field, while on Rice Research Station the total rainfall during the trial was 416 mm. Though sorghum on Rice Research Station was planted on late summer, the average temperature was higher on Rice Research Station (27°C) than in Ben Hur Research Station, (25.7°C) during the trials. Even though these difference were not remarkable, they certainly contributed to the variations in *M. sacchari* abundance between the two locations.

EPG. The host-selection process in aphids occurs as a sequence of behaviors, recognized as a sequence of events (Powell et al. 2006). Although in the actual study differences were detected in total probe and non-probing time, number of probes, proportion of individuals reaching xylem phases; feeding parameters are more significant to access host suitability (Reese et al. 2000, Brewer and Webster 2001). Indeed, these feeding parameters such as time required to first probe, time on pathway phase, and duration of sieve element feeding have been used to characterize cultivars as resistant or susceptible to aphids (Kennedy et al. 1978, Campbell et al. 1982, Alvarez et al. 2006, Caillaud and Via 2000).

In our study on genotypes PI524770 and PI643515, the percentage of aphids reaching phloem was relatively lower than on the other genotypes and pathway phase was extended, suggesting that for these genotypes there is some level of resistance in locating sieve elements (Alvarez et al. 2006, Khan et al. 2015). Studies with wheat and aphid EPG experiments also indicated the effect of cultivar associated deterrent or repellent effects (Dreyer and Jones 1982, Givovich and Niemeyer 1995, Khan et al. 2015).

High levels of 2, 4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) on wheat, interfered in the pathway phase of five cereal aphids with aphids taking longer to reach phloem

phase and lower proportion of aphids starting sieve element feeding (Argandona et al. 1983, Givovich and Niemeyer 1991, Massardo et al. 1994, Givovich and Niemeyer 1995). In our EPG studies, the differences in pre-ingestion activities, such as number of probes, time from the first probe to first phloem contact, support the argument that morphological or chemical factors outside sieve elements are affecting *M. sacchari* recognition and access to sieve elements on PI524770 and PI643515 genotypes.

Studies indicate that aphid resistance in some plants has a phloem located mechanism (Lei et al. 2001, Garzo et al. 2002, Alvarez et al. 2006, Davis and Radcliffe 2008), and committed phloem ingestion has been regarded as host plant acceptance with respect to feeding (Montllor and Tjallingii 1989, Powell et al. 2006, Pettersson et al. 2007). In our study, once the sieve elements were reached, the time spent on PI220248 was the greatest, while on PI643515 the phloem-feeding time was the lowest indicating host acceptance and rejection respectively.

Shorter duration of ingestion from sieve elements has been attributed to the relatively lower estimates of the intrinsic rate of increase for the green peach aphid, *Myzus persicae* Sulzer, on barley, *Hordeum vulgare* L., and rye, *Secale cereale* L. (Davis and Radcliffe 2008). Because of the relatively short time that the bird cherry-oat aphid, *Rhopalosiphum padi*, ingested from the sieve elements of nitrogen deficient barley, nutrient uptake was reduced resulting in reduced aphid fertility and intrinsic rate of increase (Ponder et al. 2000).

In the present work, *M. sacchari* intrinsic rate of increase on PI220248 was 1.3-fold greater than on PI643515. The differences in the mean duration of plant sap ingestion between PI220248 and PI643515 explain the decreased population parameters of *M. sacchari* on

PI643515. This difference detected in sieve elements ingestion suggests that for this genotype resistance also occurs at the phloem level.

In our study *M. sacchari* showed extended phloem salivation on PI524770. Prolonged salivation on sieve elements is an indicative of aphid's reduced ability to suppress phloem wound responses (Tjallingii 2006). Our findings, therefore, indicate that the most likely basis of resistance in PI564163 is a phloem-associated mechanism.

Host plant nutritional factors can affect aphids feeding behavior, total food consumption, and consumption rate (Mittler 1967, Mittler and Meikle 1991, Douglas 2006, Pescod et al. 2007). For instance, *M. sacchari*, spent less time ingesting phloem sap from a resistant sugarcane cultivar lacking two free essential amino acids, histidine and arginine (Akbar et al. 2014). Thus, it is likely that variations in amino acid concentrations at the phloem sap level contributed toward observed differences in aphid feeding behavior on PI643515, leading to the reduced life table parameters of *M. sacchari*. Other possibilities for the differences observed in aphid feeding behavior on sorghum genotypes include phloem-based defense and the presence of a feeding deterrents or lack of feeding stimulants in the sap of PI643515 (Caillaud and Via 2000, Zehnder et al. 2001, Zhai et al. 2017).

Effects of sorghum genotypes on two primary feeding behavior parameters of *M. sacchari* were revealed in this study. *Melanaphis sacchari* had difficulty in locating and accessing sieve elements on PI524770 and PI643515. It also showed extended salivation on PI524770 and the total duration of phloem sap ingestion was shortest on the resistant cultivar PI643515.

Use of resistant cultivars is an environmental and economically valuable management tool for aphids, especially if associated with other control tactics and reduction of pesticide

utilization (Smith 2005). Host plant resistance was especially important when *S. graminum* shifted from wheat to sorghum almost impairing sorghum production in the United States (Michels and Burd 2007), and now with *M. sacchari* shift from sugarcane to sorghum this tool recovery its importance on sorghum research.

Through a reduction in reproductive parameters and longevity, increase in juvenile mortality and inhibition of overall population parameters, genotypes PI524770, PI564163 and PI643515 were antibiotic to *M. sacchari*. The field results showed minor *Melanaphis sacchari* counts and no aphids were found on PI524770. These lines were consistently the most resistant and therefore are available to be used in the development of resistant grain sorghum hybrids.

Based on the EPG studied, *M. sacchari* had difficulty in locating and accessing sieve elements and extended salivation on phloem on genotypes PI524770 and PI564163. This indicates antixenosis in the form of deterrent factors in epidermis and mesophyll tissues. However, they did not differ in phloem feeding time from the susceptible genotypes, indicating antibiosis too. Aphids on PI643515 also had difficulty in locating and accessing sieve elements and showed shortened phloem sap ingestion indicating true antixenosis in epidermal, mesophyll, and phloem tissues.

Since there was no correlation between greenbug resistance and *M. sacchari* resistance, more studies on various sorghum sources are needed. Increasing the number of sorghum hybrids resistant to *M. sacchari* will certainly decrease the pesticide use for aphid control (Webster and Starks 1984, Hill et al. 2004), and can even enhance biological and cultural control (Smith 2005), creating a truly integrated pest management program that will benefit sorghum producers.

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CHAPTER 6. GENERAL SUMMARY

Melanaphis sacchari has become a serious pest of sorghum in the last five years, either due to a new genotype in the population or due to a foreign introduction into the United States. In order to aid the integrated pest management program for this pest, life table and feeding behavior studies were conducted to better understand *M. sacchari* plant interactions and population dynamics. Laboratory experiments were conducted to determine *M. sacchari* population growth and thermal thresholds on sorghum under six different constant temperatures. These studies calculated *M. sacchari* upper and lower thermal thresholds at 8.6°C and 37.8°C and optimal development at 28.3°C. These developmental thresholds data suggest *M. sacchari* can tolerate elevated temperatures and indicate greater *M. sacchari* population increments during hotter periods.

The studies conducted in this dissertation indicate the *M. sacchari* sorghum genotype is more adapted to exploit sorghum. Life table studies indicated that this clone performs better on *Sorghum* spp. than on sugarcane and can colonize rice and wheat. Though this study indicated that population growth was low on wheat and rice, these plant species are in abundance in the Louisiana agroecoscape, and therefore wheat and rice could be used as bridging species for *M. sacchari* while sorghum is not available. Life table studies indicated that *M. sacchari* populations can rapidly increase on sugarcane and Johnsongrass. Johnsongrass is a weed species in sorghum fields and surroundings and Louisiana has one of the biggest sugarcane areas of the United States. These plants are available all year, so *M. sacchari* can increase populations on these plants and later migrate to sorghum. Thus, we demonstrate that there is the potential for multiple crop

use within the agroecoscape by *M. sacchari* which could facilitate migration between sorghum, sugarcane, wheat, and rice production.

Using the Electronic Penetration Graph (EPG) technique we demonstrated that *M. sacchari* can establish sustained feeding on sorghum, Johnsongrass, wheat, and rice. On maize and sweetpotato, *M. sacchari* did not establish sustained feeding and the lowered life table parameters observed were a consequence of *M. sacchari* starvation. On rice and wheat, phloem located defenses, combined with difficulties in locating and accepting sieve elements as observed using EPG, explained the lowered fitness of *M. sacchari*. *Melanaphis sacchari* was able to quickly reach and sustain feeding in the sieve elements of sorghum and Johnsongrass explaining the high fitness of *M. sacchari* on these plants.

This work has provided the basis for the potential use of sorghum plant introductions in breeding programs to improve *M. sacchari* host plant resistance in sorghum. From the 12 sorghum genotypes studied in laboratory, three genotypes, PI453951, PI220248, and PI 456889, were the most susceptible and should be avoiding in breeding schemes. Greenhouse trials confirmed that PI453951, PI220248, and PI456689 were highly susceptible to *M. sacchari*. In general, field trials had low aphid infestations and only genotype PI220248 had high numbers of *M. sacchari*.

Laboratory no-choice studies indicated that genotypes PI524770, PI564163, and PI643515, are antibiotic to *M. sacchari*. Results of aphid multiplication in greenhouse studies confirmed laboratory results. Field results validated greenhouse and laboratory studies. Consistently PI524770, PI564163, and PI643515 were resistant to *M. sacchari*, and have the potential to be used in the development of resistant grain sorghum hybrids.

Based on the EPG study, *M. sacchari* had difficulty in locating and accessing sieve elements and in extended salivation in phloem on genotypes PI524770, PI643515, and PI643515. This indicates antixenosis in the form of deterrent factors in epidermal and mesophyll tissues. However, they did not differ from the susceptible genotypes in phloem-feeding duration, indicating that antibiosis may also play a role in these genotypes. The shortened phloem sap ingestion in PI643515 suggests feeding deterrence in phloem tissues.

Future studies to elucidate what are the mechanism that underlies the heat tolerance of *M. sacchari*, and if there is any fitness tradeoff associated with this characteristic would increase the understanding of this pest biology. Since the literature reports of *M. sacchari* host range includes other common plants found in the United States, host plant interaction with other plants should also be investigated. Additionally, specific salivary compounds of this *M. sacchari* genotype can be a major factor in the successful exploitation of sorghum plants. Finding out which of these factors may contribute to this host shift and if they differ from sugarcane only clones would help explain the change in hosts. Studies should continue to investigate sources of resistance to *M. sacchari* in sorghum and should include investigations into specific factors affecting *M. sacchari* susceptibility. Futures studies will increase the understanding of *M. sacchari* on sorghum in the United States and will enable the creation of a truly integrated pest management program.

The findings of this study will assist in planning control measures for *M. sacchari* on sorghum and provide sorghum resistant sources to be used in breeding programs. Before economic thresholds can be developed, information on population growth under simulated conditions is needed, therefore the thermal thresholds and population parameters observed in

the present study are essential for an effective integrated pest management. Spatial planning is also an important component of integrated pest management. The data provided in this study about *M. sacchari* population dynamics, as well as the evidence that *M. sacchari* can use other crops as bridging species provided the information needed to prevent or anticipate large colonizing populations on sorghum fields. PI524770, PI564163, and PI643515 were consistently resistant to *M. sacchari* and can be used to develop commercial hybrids, increasing integrated management options.

VITA

Monique Ferreira de Souza, from Brazil, got her Master in Crop Production, after receiving her bachelor degree in Agronomy from the Federal University of Uberlandia. During her M.S., she studied the plant-insect-parasitoid tri-trophic interaction. As her interest in entomology grew, she decided to enter the Department of Entomology at Louisiana State University. Upon completion of her Ph.D.'s degree, she will continue her studies and research in entomology.