

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Dissertations and Student Research in
Entomology

Entomology, Department of

Spring 5-2022

First Line of Defense: Role of Surface Waxes in Sorghum Defense Against Aphids

Juan David Betancurt Cardona
University of Nebraska-Lincoln

Follow this and additional works at: <https://digitalcommons.unl.edu/entomologydiss>



Part of the [Agriculture Commons](#), and the [Entomology Commons](#)

Betancurt Cardona, Juan David, "First Line of Defense: Role of Surface Waxes in Sorghum Defense Against Aphids" (2022). *Dissertations and Student Research in Entomology*. 80.
<https://digitalcommons.unl.edu/entomologydiss/80>

This Article is brought to you for free and open access by the Entomology, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Dissertations and Student Research in Entomology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

FIRST LINE OF DEFENSE: ROLE OF SURFACE WAXES IN SORGHUM DEFENSE
AGAINST APHIDS

by

Juan David Betancurt Cardona

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Entomology

Under the Supervision of Professor Joe Louis

Lincoln, Nebraska

May 2022

FIRST LINE OF DEFENSE: ROLE OF SURFACE WAXES IN SORGHUM DEFENSE AGAINST APHIDS

Juan David Betancurt Cardona, M.S.

University of Nebraska, 2022

Advisor: Joe Louis

Since 2013, the sugarcane aphid (SCA; *Melanaphis sacchari*) has become a devastating pest in sorghum (*Sorghum bicolor*) in the United States. Efforts to mitigate this pest and contain its rapid population growth have increased recently. Host plant resistance (HPR) is an effective and environmentally friendly strategy to reduce aphid populations in this crop. Many factors make SCA a challenging pest to control. For example, SCA can overcome drastic climatic changes, which allows them to successfully colonize sorghum plants at different developmental stages. Epicuticular waxes (EW) constitute the first point of contact between plants and their environment. EW differ in their structure and composition at different developmental stages in sorghum. In addition, EW are known to be involved in protecting plants from external stresses. However, the role of EW in sorghum-SCA interactions is not fully understood. To elucidate the role of EW in the SCA-sorghum interactions, we used the Electrical Penetration Graph (EPG) technique to monitor the SCA feeding behavior at different developmental stages of sorghum BTx623 plants and, in sorghum wild-type and bloomless (wax-depleted) plants. Additionally, through no-choice and choice assays, we determined whether the EW influence the SCA

survival (antibiosis) and behavior (antixenosis) in sorghum plants and how the wax composition is related with plants responses against SCA. We found that aphids prefer to feed longer in the sieve elements of six-week-old plants compared to two-week-old plants. However, the SCA proliferation was higher on two-week-old plants compared to six-week-old plants. Our EPG results revealed that the aphids spent more time feeding in the xylem phase and preferred to settle in the bloomless plants compared to wild-type plants. The abundance of α -amyirin and isoarborinone, both belonging to the triterpenoid family, increased after aphid infestation in six-week-old plants compared to six-week-old plants. The total amount of 16-monoacylglycerols and 32C-alcohols was higher in bloomless plants compared to the wild-type plants. Results from this study helped us to further understand the role of epicuticular waxes play in aphid-plant interactions and will contribute to the understanding of the mechanisms involved in plants defenses and their association with waxes in plants.

ACKNOWLEDGMENTS

First, I must profoundly thank my thesis and research advisor Dr. Joe Louis for his great advice, support, and mentoring work throughout my master's program. Even though times were not easy during COVID-19, his great support and patience allowed me to move forward the program. I would also like to thank my committee members Drs. Scott Sattler and Kyle Koch for each of their input and guidance throughout this project.

I would like to especially thank Dr. Lucas Busta and the members of his laboratory for their contributions to this research. This research would not have been possible without the constant encouragement, training, and friendship of Dr. Sajjan Grover who shared much of his experience as a student and as a member of the laboratory with me.

I also want to thank those people who supported me during this experience and encouraged me to continue building my professional future, especially to Gabriela Carmona, Vilma Montenegro, Mia Luong, Heena Puri and finally, my friends and great leaders Osler Ortéz and Veronica Hotovy.

I am grateful to all the faculty and staff in the entomology department for their help. To our head department Dr. John Ruberson for his great accompaniment throughout the hard times.

Finally, I want to thank God for opening the doors and allowing me to grow in life through this incredible experience. To my mom, my dad, and my brother, the three biggest reasons I must fight to find the best version of myself and make them proud with every step I take, I love you with my soul. To all those people that perhaps I could forget on this page, thank you very much for everything.

TABLE OF CONTENTS

CHAPTER 1: Literature review.....	1
Sorghum	1
Insect pests of Sorghum	1
Sugarcane Aphid (<i>Melanaphis sacchari</i>).....	3
Plant defenses	5
First line of defense – Waxes	6
Sorghum waxes change across different developmental stages.....	7
Electrical Penetration Graph to monitor aphid feeding behavior.....	8
Significance of the study, objectives, and hypotheses	9
References	11
CHAPTER 2: Different developmental stages of sorghum (<i>Sorghum bicolor</i>) impacts sugarcane aphid (<i>Melanaphis sacchari</i>) population growth and feeding behavior....	16
Abstract	16
Introduction	18
Materials and methods	22
Results	26
Discussion	28
Acknowledgments.....	31
References	32
CHAPTER 3: Role of epicuticular waxes in sorghum resistance to sugarcane aphids (SCA) (<i>Melanaphis sacchari</i>)	45
Abstract	45
Introduction	47
Materials and methods	49
Results	53
Discussion	55
Acknowledgments.....	57
References	58

LIST OF FIGURES

Figure 1.1: EPG setup to understand the feeding behavior of aphids on host plants	15
Figure 2.1: Total number of SCA adults and nymphs per plant in two-week-old and six-week-old BTx623 plants	37
Figure 2.2: EPG waveforms representing the SCA aphid feeding behavior in two-week-old and six-week-old in sorghum BTx623 plants	38
Figure 2.3: SCA feeding behavior parameters in six-week-old and two-week-old sorghum BTx623 plants	39
Figure 2.4: Time taken by SCA to first probe, to reach first sieve elements and total number of potential drops in six-week-old and two-week-old sorghum BTx623 plants ..	40
Figure 2.5: Detailed sieve element phase analysis	41
Figure 2.6: Abundance of each of the wax components present in the leaf sample between two-week-old plants and six-week-old BTX623 plants	44
Figure 3.1: Total number of SCA adults and nymphs per plant in wild-type and bloomless plants	61
Figure 3.2: Proportion of SCA settled on sorghum wild-type and bloomless plants at 1 h, 6 h and 24 h.....	62
Figure 3.3: Sugarcane aphid (SCA) feeding behavior parameters in wild-type and bloomless plants	63
Figure 3.4: Abundance of each of the epicuticular wax components present in the leaf sample between sorghum wild-type and bloomless plants	64

CHAPTER 1: Literature review

Sorghum

Sorghum (*Sorghum bicolor* L. Moench [Poaceae]) is one of the most important crops grown in the world and ranked in fifth place preceded only by wheat, rice, maize, and barley. Although sorghum is grown in other countries for human consumption it is predominantly used for animal feed in the United States (Morais Cardoso et al., 2015). Global sorghum production has grown recently due to its high nutritional content, and it could also serve as a source of high nutritional quality food for daily consumption. Since sorghum also generate a large amount of biomass, it is also grown in some parts of the world for production of low-cost feedstock for ethanol production (Ferreira et al., 2009; Guo et al., 2011). Sorghum is one of the most drought tolerant cereals, as it is able to roll its leaves to reduce water loss due to respiration. Under high drought stress, it turns to a dormant stage to avoid death by desiccation. In addition, the leaves of sorghum plants are protected by a cuticle to reduce evapotranspiration (Ramatoulaye et al., 2016).

Insect pests of Sorghum

Since sorghum is a widely used crop, the presence of pests has had a greater impact with the development of new cultivars and the appearance of new pests that overcome the natural defense mechanisms of this crop. Insect pests have caused losses of up to \$250 million in the USA (Sharma et al., 1997) and cause up to 90% annual losses for different sorghum cultivars in southern part of the USA (Okosun et al., 2021). Higher seeding rates and its tillering ability make stand losses to soil insects less severe than in corn. However, insect pests have been able to colonize sorghum very effectively, specializing in different

plant structures: roots (Lesser cornstalk borer; *Elasmoplapus lignosellus*), stem (Sugarcane beetle; *Euetheola humilis rugiceps*), leaves (aphids), whorl region (worms and mites), panicle and seeds (sorghum midge; *Stenodiplosis sorghicola*) (Buntin 2012). To date, 150 insect species have been described as a pest in sorghum, most of them occurring in Africa. In North America, the fall armyworm (*Spodoptera frugiperda*), corn borer (*Ostrinia furnacalis*), sorghum midge (*Contarinia sorghicola*) and the greenbug (*Schizaphis graminum*) are the major pests (Guo et al., 2011).

Aphids include a large group of insects in the superfamily Aphidoidea (Hemiptera). To date, more than 250 aphid species have been described as a pest of cultivated plants (Nalam et al., 2018). Aphids are a diverse group of insects, which have many structures morphologically adapted to the environment that varies in different groups. One of the most unique is the presence of a paired siphunculi (cornicles) through which they release an alarm pheromone (Sorensen 2009). Aphids reproduce parthenogenically, an asexual mode of reproduction that combine a short generation time to increase their populations in a short period of time. Additionally, aphids can produce winged morphs to facilitate dispersion to plants and a unique feeding behavior that makes them an important pest worldwide (Nalam et al., 2018).

Aphids are piercing-sucking class of insects that have adapted to feed from sieve elements (Louis & Shah 2013). Their mouthparts have a modified slender stylet, which is used to penetrate the plant tissue and ingest the nutrients from the sieve elements (Douglas 2003). Aphid saliva is critical to the progress of the stylets through the plant tissue. Saliva is produced in paired salivary glands and secreted via the salivary canal (Douglas 2003). When the aphids penetrate the plant tissue they release a gelling saliva,

which lubricate and harden the cellular walls to form a sheath and facilitates the stylet movement. Subsequently, when the aphid starts the feeding activity, they release watery saliva through the stylet. The watery saliva contains components that allow the aphid to prevent and possibly reverse phloem obstruction, thus allowing the insect to feed continuously from a single sieve element (Louis & Shah 2013; Nalam et al., 2018).

Sugarcane Aphid (*Melanaphis sacchari*)

With the increase in the production of sorghum in the United States, the pressure from newly discovered pests in this crop has been increasing. Such is the case of sugarcane aphid (SCA; *Melanaphis sacchari*) (Hemiptera:Aphididae), which has recently become one of the most damaging insect pests of sorghum in the USA (Okosun et al., 2021). Although SCA was already considered a pest in Africa, Asia, Australia, Central and South America, and it was previously not identified as a pest in sorghum in the USA (Okosun et al., 2021). Since 2013, SCA became a pest on sorghum by rapidly increasing their populations in Mexico. Quickly, SCA reached the Gulf of Texas and Louisiana in the same year (Bowling et al., 2016; Villanueva et al., 2014), and finally in 2015 this pest was reported in 17 U.S. states where sorghum was grown.

The two biotypes of SCA described as a pest in the United States are MLL-D and MLL-F lineage (Harris-Shultz et al., 2019) and are predominantly: 1) anholocyclic - sexual reproduction is not known to occur (Holland et al., 2003); 2) Parthenogenic, when the production of offspring occurs in the absence of any male genetic contribution (Awruh 2015); and 3) viviparous (live-bearing), which consists in developing embryos retained in or the body of a parent (Brewer et al., 2017; Hagan 1948). The color varies in different

ranges from gray to yellow. It also has the dark cornicles, tarsi, and antennae, a unique characteristic of this species (Bowling et al., 2016).

Sugarcane aphid, like other aphids, consumes the nutrients from the plant tissue, locating their host via olfactory and/or visual cues (Nalam et al., 2018). Using a sap/sucking structure that is inserted in the plant tissue (slender stylet) (Akbar et al., 2014; Zogli et al., 2020) aphids' uptake the nutrients by removing the plant sap from the xylem (Singh et al., 2004) and phloem tissues (Tetreault et al., 2019).

Abundant populations of SCA on sorghum plants caused significant yield losses by negatively impacting plant vigor, purple leaf discoloration of seedlings and head emergence, and the presence of abundant honeydew that affected the harvesting effectiveness. However, the effectiveness of SCA colonization on host plants depend on many factors, including population dynamics and the infestation duration (Singh et al., 2004).

The current management strategies to control SCA involve several approaches. It includes the use of chemical practices, such as the use of insecticides (e.g., Transform, sulfoximines) and cultural practices (early inspection of the SCA activity in the field and alternation of the planting date) (Lofton & Arnall 2017). Additional practices involve the development of sorghum hybrids that are resistant to SCA (Knutson et al., 2016), incorporation of natural enemies of SCA in sorghum fields (e.g., Lady beetles, dusky lady beetles, lacewings, hoverflies, parasitoid wasps [(Knutson et al., 2016; Bowling et al., 2016)]. Finally, an additional potential approach for SCA management is the implementation of host plant resistance, known for its ease of use, potential affordability, and compatibility with natural enemies (Bowling et al., 2016).

Plant defenses

Plants do not have mobile defense strategies (sessile organisms) comparable to those of other living beings. However, plants have evolved an endless number of innate immune and defensive responses, both chemical and physical, that allow them to protect themselves from their natural enemies (Kaloshian & Walling 2016). Plant resistance can be classified into three different categories: Antibiosis, antixenosis and tolerance.

Antibiotic responses affect the biology (survival, development, and fecundity) of the insect, antixenotic responses alters the insect behavior leading to the insect non-preference to a specific host and tolerance responses are defined as the capacity of a plant to withstand and recover from any insect injury (Smith & Clement 2012; Nalam et al., 2018).

Plants can develop different sort of responses within the categories mentioned above. These responses, known as “mechanisms”, describe the chemical or morphological processes that explain plant negative responses to an insect attack. Structural barriers (e.g., plant pubescence, trichomes) and allelochemicals (glucosinolates, alkaloids, terpenoids) constitutes the direct defense of the plant, whereas the indirect defenses consist of the production of volatile organic compounds released by the plant that attract natural enemies of the herbivore (i.e., Herbivore-Induced Plant Volatiles (HIPV)) or affects the oviposition of the insect (Smith & Clement 2012).

Studies have identified numerous defense strategies in sorghum against SCA. Tetreault et al (2019) identified that several nucleotide-binding-site, leucine-rich repeats (NBS-LRR) and disease resistance genes were upregulated in the sorghum SCA-resistant RTx2783 line. Additionally, the resistance line showed both antixenosis and antibiosis-mediated

resistance to SCA. Moreover, Kiani & Szczepaniec (2018) found that a higher number of differentially expressed genes in young sorghum resistant plants correlated to the induction of hormone-signaling pathways, pathways coding for secondary metabolites, and plant-pathogen interactions.

First line of defense – Waxes

The surface of the plants (cuticle) provides the first point of contact in insect-plant interactions. Cuticle waxes could contribute to plant responses after aphid attack. It is known that some plant defenses (direct or indirect) are related with leaf wax composition and structure upon herbivory (Nalam et al., 2018).

Waxes in the plant cuticle are the second major component covering the plant surface. They are present in the stem, leaves, petals, and fruits (Khattab 2007; Wojcicka 2013; Jetter et al., 2008). Two main layers compose the protective wax coating in the leaves – the intracuticular and epicuticular layers (Shepherd et al., 1999). The main function of the wax coating in the cuticle is to prevent desiccation in drought conditions. In sorghum, waxes may help to reduce water loss in dry environments. However, this structure is also involved in important functional and structural parts of the plant (Eigenbrode & Espelie 1995).

The epicuticular waxes play an important role in protecting aerial organs (Wojcicka 2014). This structure is formed by complex mixture of long chain aliphatic and cyclic components, including n-alkanes, wax esters, free fatty alcohols, and free fatty acids, as well as low levels of terpenoids, sterols, flavonoids, and phenolic substances (Busta et al., 2021; Wojcicka & Agnieszka 2015, Kumar et al., 2017; Griffiths et al., 2000).

Additionally, this layer also protects the plant from UV-B and pathogen invasion, and functions as allelochemical, influencing insect behavior and biology (Wojcicka & Agnieszka 2015). Considering that epicuticular waxes form the first point of contact and possibly the first barrier in defense against aphids, many properties of epicuticular waxes can generate a behavioral and/or biological response in aphids, instantaneously modifying the insertion activity of the stylet in host plants (Verdugo et al., 2007), aphid adherence/movement to the plant and probing behavior (Shepherd et al., 2000). Changes in the nature of the chemical composition and the amount of epicuticular waxes can vary between species, genotypes, plant structures and age (Busta et al., 2021). Variations in this structure could influence the final interaction response from plants upon herbivory (Eigenbrode & Espelie 1995).

Sorghum waxes change across different developmental stages

Waxes are important in several physiological, morphological, and behavioral mechanisms in sorghum. A recent study demonstrated that variations in sorghum wax surface characteristics can be associated with the juvenile-to-adult transition life cycle (Busta et al. 2021). The study has also found drastic differences in the wax leaf coverage and leaf wax composition between juvenile sorghum leaves (~14 days old) and adult sorghum leaves (~40 days old) (Busta et al. 2021). Interestingly, SCA has been found to overcome the climate challenges and drastic weather conditions colonizing sorghum in vegetative stages in the southern U.S. where they can overwinter on non-crop vegetation (i.e., Johnsongrass), while colonizing sorghum in reproductive stages in the northern states after wind-aided migration (Kiani & Szczepaniec 2018). Although the function and structure of the epicuticular waxes in sorghum plants and their interaction with the

environment are widely known, little is known about the role that epicuticular wax components may have in altering the biology and behavior of SCA.

Electrical Penetration Graph to monitor aphid feeding behavior

As mentioned previously, aphids are sap-sucking insects, and monitoring its feeding behavior is a challenging process since they feed on a substrate that is not directly observable. The Electrical Penetration Graph (EPG) technique has become the most significant tool for the monitoring of aphid feeding behavior (Varsani et al., 2019). The EPG system consist of two electronic components, a voltage source and an input resistor that are electrically connected each other (Walker 2000) in which the insect and a plant are incorporated to this simple circuit through a copper wire. One wire is connected to the plant (plant electrode) and the other to the insect (insect electrode); the insect is glued in the dorsum with a small drop of an electrically conductive adhesive (Fig. 1). Electrical Penetration Graph (EPG) measures small fluctuations in the voltage within the simple circuit described above. In summary, the changes in the membrane potentials at the extra and intracellular level of the cellular interstices are the key for the measurement of the different feeding behaviors. In addition, the insertion and secretion of the two salivary components through the aphid stylets also influence the voltage source of the circuit (Walker 2000).

The EPG measurements are usually then transformed in distinctly and recognizable patterns of fluctuating voltage known as waveforms. The different patterns or waveforms correlate to different feeding behaviors of the insect (Tjallingii 2006. The most frequent identified phases are the pathway, xylem, phloem, and non-probing phases (Tjallingii 2006; Wojcicka & Agnieszka 2015; Grover et al., 2019; Kindt et al., 2003; Garzo et al.,

2016), however, several patterns detected for the EPG could be interpreted in different feeding activities (e.g., salivation activity, ingestion of the phloem sap activity, probing, among others). The use of EPG has been validated in many studies as a useful tool for the biological validation of several process in insect-plant interactions, as well the resistance levels of plants against herbivory (Mutti et al., 2008; Tetreault et al., 2019; Grover et al., 2020; Pegadaraju et al., 2007; Diaz-Montano et al., 2007; Koch et al., 2015; Koch et al., 2016)

Significance of the study, objectives, and hypotheses

It is critical to understand the mechanisms underlying the first line of defense in sorghum plants and aphid feeding behavior for developing novel pest management strategies.

Elucidating these interactions between the leaf epicuticular waxes present in sorghum as the first point of contact or first defense barrier against the SCA would allow, primarily to enhance the knowledge of the ecology and biology of the pest and secondly, the development of better approaches for the protection of sorghum in the U.S. through more environmentally friendly strategies. To identify these unknown mechanisms and improve the knowledge about the ecology of the SCA, this study aims to understand the role of surface waxes in sorghum resistance to SCA. To accomplish this, the first objective is to use the Electrical Penetration Graph (EPG) to monitor the feeding behavior of SCA on different developmental stages of sorghum (two-week vs six-week-old sorghum plants), and the second objective is to quantify and correlate the wax composition and resistance/susceptibility to SCA in two-week and six-week-old sorghum plants. We hypothesize that older plants will have more epicuticular wax components and provide enhanced resistance to aphids compared to younger plants. Additionally, the number of

aphids will be higher on those plants without waxes. The relative abundance of components and coverage of waxes will therefore be a determining factor in the sorghum resistance and the composition of waxes will be closely linked to the population growth of the aphid in the plant (susceptibility/resistance).

References

- Akbar, W., Showler, A. T., Reagan, T. E., Davis, J. A., & Beuzelin, J. M.** (2014). Feeding by sugarcane aphid, *Melanaphis sacchari*, on sugarcane cultivars with differential susceptibility and potential mechanism of resistance. *Entomologia Experimentalis et Applicata*, 150(1), 32-44.
- Awruch, C. A.** (2015). Reproduction strategies. In *Fish physiology* (Vol. 34, pp. 255-310).
- Bowling, R. D., Brewer, M. J., Kerns, D. L., Gordy, J., Seiter, N., Elliott, N. E., Buntin, G.D., Way, M.O., Royer, T.A., Biles, S., & Maxson, E.** (2016). Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. *Journal of Integrated Pest Management*, 7(1), 12; 1-13.
- Brewer, M. J., Gordy, J. W., Kerns, D. L., Woolley, J. B., Rooney, W. L., & Bowling, R. D.** (2017). Sugarcane aphid population growth, plant injury, and natural enemies on selected grain sorghum hybrids in Texas and Louisiana. *Journal of economic entomology*, 110(5), 2109-2118.
- Buntin, G. D.** (2012). *Grain Sorghum Insect Pests and Their Management*. University of Georgia Cooperative Extension. Bulletin, 1283.
- Busta, L., Schmitz, E., Kosma, D. K., Schnable, J. C., & Cahoon, E. B.** (2021). A co-opted steroid synthesis gene, maintained in sorghum but not maize, is associated with a divergence in leaf wax chemistry. *Proceedings of the National Academy of Sciences*, 118(12).
- Diaz-Montano, J., Reese, J. C., Louis, J., Campbell, L. R., & Schapaugh, W. T.** (2007). Feeding behavior by the soybean aphid (Hemiptera: Aphididae) on resistant and susceptible soybean genotypes. *Journal of Economic Entomology*, 100(3), 984-989.
- Douglas, A. E.** (2003). The nutritional physiology of aphids. *Advances in insect physiology*, 31(31), 73-140.
- Eigenbrode, S. D., & Espelie, K. E.** (1995). Effects of plant epicuticular lipids on insect herbivores. *Annual review of entomology*, 40(1), 171-194.
- Ferreira, Sila & Luparelli, Paola & Schieferdecker, Maria Eliana & Vilela, Regina.** (2009). Cookies sem glúten a partir da farinha de sorgo. *Archivos Latinoamericanos de Nutrición*. 59. 433-440.
- Garzo, E., Moreno, A., Hernando, S., Mariño, V., Torne, M., Santamaria, E., ... & Fereres, A.** (2016). Electrical penetration graph technique as a tool to monitor the early stages of aphid resistance to insecticides. *Pest management science*, 72(4), 707-718.
- Griffiths, D. W., Robertson, G. W., Shepherd, T., Birch, A. N. E., Gordon, S. C., & Woodford, J. A. T.** (2000). A comparison of the composition of epicuticular wax from red raspberry (*Rubus idaeus* L.) and hawthorn (*Crataegus monogyna* Jacq.) flowers. *Phytochemistry*, 55(2), 111-116.

- Grover, S., Varsani, S., Kolomiets, M. V., & Louis, J.** (2020). Maize defense elicitor, 12-oxo-phytodienoic acid, prolongs aphid salivation. *Communicative & Integrative Biology*, 13(1), 63-66.
- Grover, S., Wojahn, B., Varsani, S., Sattler, S. E., & Louis, J.** (2019). Resistance to greenbugs in the sorghum nested association mapping population. *Arthropod-Plant Interactions*, 13(2), 261-269.
- Guo, C., Cui, W., Feng, X., Zhao, J., & Lu, G.** (2011). Sorghum insect problems and Management. *Journal of integrative plant biology*, 53(3), 178-192.
- Hagan, H. R.** (1948). A brief analysis of viviparity in insects. *Journal of the New York Entomological Society*, 56(1), 63-68.
- Harris-Shultz, K., Armstrong, S., & Jacobson, A.** (2019). Invasive cereal aphids of North America: Biotypes, genetic variation, management, and lessons learned. *Trends in Entomology*, 15, 99-122.
- Holland, J. B., Nyquist, W. E., Cervantes-Martínez, C. T., & Janick, J.** (2003). Estimating and interpreting heritability for plant breeding: an update. *Plant breeding reviews*, 22, 9-112.
- Jetter, R., Kunst, L., & Samuels, A. L.** (2008). Composition of plant cuticular waxes. *Biol. Plant Cuticle*, 23, 145-181.
- Kaloshian, I., & Walling, L. L.** (2016). Hemipteran and dipteran pests: effectors and plant host immune regulators. *Journal of Integrative Plant Biology*, 58(4), 350-361.
- Khattab, Hemmat.** (2007). The defense mechanism of cabbage plant against phloem-sucking aphid (*Brevicoryne brassicae* L.). *Australian Journal of Basic and Applied Sciences*. 1(1), 56-62.
- Kiani, M., & Szczepaniec, A.** (2018). Effects of sugarcane aphid herbivory on transcriptional responses of resistant and susceptible sorghum. *BMC genomics*, 19(1), 1-18.
- Kindt, F., Joosten, N. N., Peters, D., & Tjallingii, W. F.** (2003). Characterization of the feeding behavior of western flower thrips in terms of electrical penetration graph (EPG) waveforms. *Journal of Insect Physiology*, 49(3), 183-191.
- Knutson, A., Bowling, R., Brewer, M., Bynum, E., & Porter, P.** (2016). The sugarcane aphid: management guidelines for grain and forage sorghum in Texas. Texas A&M AgriLife Extension, College Station, TX. NTO-035.
- Koch, K. G., Chapman, K., Louis, J., Heng-Moss, T., & Sarath, G.** (2016). Plant tolerance: a unique approach to control hemipteran pests. *Frontiers in Plant Science*, 7, 1363.
- Koch, K. G., Palmer, N., Stamm, M., Bradshaw, J. D., Blankenship, E., Baird, L. M., Sarath, G., & Heng-Moss, T. M.** (2015). Characterization of greenbug feeding behavior and aphid (Hemiptera: Aphididae) host preference in relation to resistant and susceptible tetraploid switchgrass populations. *Bioenergy Research*, 8(1), 165-174.

- Kumar, S., Singh, Y.P., Singh, S.P. et al.** (2017). Physical and biochemical aspects of host plant resistance to mustard aphid, *Lipaphis erysimi* (Kaltenbach) in rapeseed-mustard. *Arthropod-Plant Interactions* 11, 551–559.
- Leandro de Morais Cardoso, Soraia Silva Pinheiro, Hércia Stampini Duarte Martino & Helena Maria Pinheiro-Sant'Ana** (2015): Sorghum (*Sorghum bicolor*): Nutrients, Bioactive Compounds, and Potential Impact on Human Health. *Critical reviews in food science and nutrition*, 57(2), 372-390.
- Lofton, J., & Arnall, D. B.** (2017). Agronomic Management Practices for Controlling Sugarcane Aphids. PSS- 2179
- Louis, J., & Shah, J.** (2013). *Arabidopsis thaliana*—*Myzus persicae* interaction: shaping the understanding of plant defense against phloem-feeding aphids. *Frontiers in Plant Science*, 4, 213.
- Mutti, N. S., Louis, J., Pappan, L. K., Pappan, K., Begum, K., Chen, M. S., Park, Y., Dittmer, N., Marshall, J., Reese, J.C., & Reeck, G. R.** (2008). A protein from the salivary glands of the pea aphid, *Acyrtosiphon pisum*, is essential in feeding on a host plant. *Proceedings of the National Academy of Sciences*, 105(29), 9965-9969.
- Nalam, V., Louis, J., & Shah, J.** (2019). Plant defense against aphids, the pest extraordinaire. *Plant Science*, 279, 96-107.
- Okosun, O. O., Allen, K. C., Glover, J. P., & Reddy, G. V.** (2021). Biology, ecology, and management of key sorghum insect pests. *Journal of Integrated Pest Management*, 12(1), 4.
- Pegadaraju, V., Louis, J., Singh, V., Reese, J. C., Bautor, J., Feys, B. J., Graeme, C., Parker, J.E., & Shah, J.** (2007). Phloem-based resistance to green peach aphid is controlled by *Arabidopsis PHYTOALEXIN DEFICIENT4* without its signaling partner *ENHANCED DISEASE SUSCEPTIBILITY1*. *The Plant Journal*, 52(2), 332-341.
- Ramatoulaye, F., Mady, C., & Fallou, S.** (2016). Production and use sorghum: a literature review. *Journal of Nutritional Health & Food Science*, 4(1), 1-4.
- Sharma, H. C., Singh, F., & Nwanze, K. F.** (1997). Plant resistance to insects in sorghum. International Crops Research Institute for the Semi-Arid Tropics.
- Shepherd, T., Robertson, G. W., Griffiths, D. W., & Birch, A. N. E.** (1999). Epicuticular wax composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeus* L.). *Phytochemistry*, 52(7), 1239-1254.
- Shepherd, T., Robertson, G. W., Griffiths, D. W., & Birch, A. N. E.** (2000). Plants and aphids: the chemical ecology of infestation. Scottish Crop Research Institute Annual Report 1999, 2000, 122-124.
- Singh, B. U., Padmaja, P. G., & Seetharama, N.** (2004). Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. *Crop Protection*, 23(9), 739-755.

- Smith, C. M., & Clement, S. L.** (2012). Molecular bases of plant resistance to arthropods. *Annual review of entomology*, 57, 309-328.
- Sorensen, J. T.** (2009). Aphids. In *Encyclopedia of insects*. Academic Press. (pp. 27-31)
- Tetreault, H. M., Grover, S., Scully, E. D., Gries, T., Palmer, N. A., Sarath, G., Louis, J., & Sattler, S. E.** (2019). Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphid (*Melanaphis sacchari*). *Frontiers in plant science*, 10, 145.
- Tjallingii, W. F.** (2006). Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of experimental botany*, 57(4), 739-745.
- Varsani, S., Grover, S., Zhou, S., Koch, K. G., Huang, P. C., Kolomiets, M. V., Williams, W.P., Heng-Moss, T., Sarath, G., Luthe, D.S, Jander, G., & Louis, J.** (2019). 12-Oxo-phytodienoic acid acts as a regulator of maize defense against corn leaf aphid. *Plant Physiology*, 179(4), 1402-1415.
- Verdugo, J. A., Astudillo, L. A., & Ramírez, C. C.** (2007). Effect of epicuticular waxes of poplar hybrids on the aphid *Chaitophorus leucomelas* (Hemiptera: Aphididae). *Journal of applied entomology*, 131(7), 486-492.
- Villanueva, R. T., & Sekula, D.** (2014). A new pest of sorghum: The sugarcane aphid. In 20th Annual Rio Grande Valley Cotton & Grain Pre-Plant Conference. counties. AgriLife.
- Walker, G. P.** (2000). A beginner's guide to electronic monitoring of homopteran probing behavior. Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD, 14-40.
- Wójcicka, A.** (2013). Importance of epicuticular wax cover for plant/insect interactions: experiments with cereal aphids. *Pol. J. Ecol*, 61(1), 183-186.
- Wójcicka, A.** (2014). Changes in pigment content of triticale genotypes infested with grain aphid *Sitobion avenae* (Fabricius) (Homoptera: Aphididae). *Acta Biologica Cracoviensia. Series Botanica*, 56(1).
- Wójcicka, A.** (2015). Surface waxes as a plant defense barrier towards grain aphid. *Acta Biologica Cracoviensia s. Botanica*, 57(1).
- Zogli, P., Pingault, L., Grover, S., & Louis, J.** (2020). Ento (o) mics: the intersection of 'omic' approaches to decipher plant defense against sap-sucking insect pests. *Current Opinion in Plant Biology*, 56, 153-161.

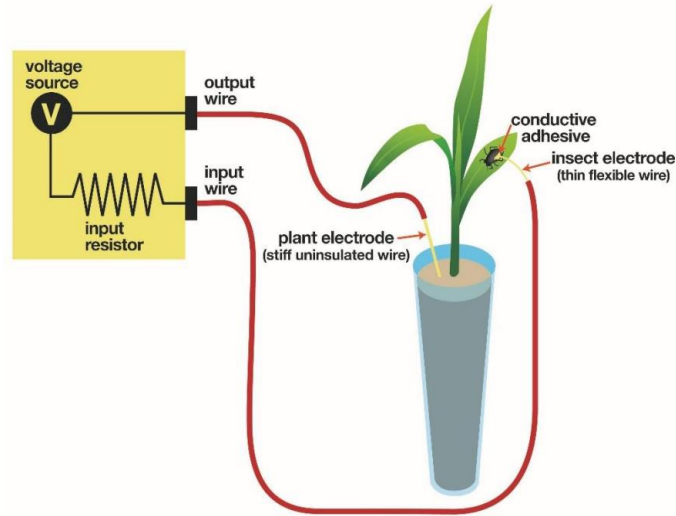


Figure 1.1

EPG setup to understand the feeding behavior of aphids on host plants. Copper wire inserted into the soil of the potted plant acts as the plant electrode. A very thin gold wire is attached to the dorsum of the aphid using silver conductive paint will serve as the insect electrode. Different waveforms/signals are generated once the aphid starts feeding on the host plant (Nalam et al., 2019). Illustration by Nick Sloff.

CHAPTER 2

Different developmental stages of sorghum (*Sorghum bicolor*) impacts sugarcane aphid (*Melanaphis sacchari*) population growth and feeding behavior

Abstract

Sugarcane aphid (SCA; *Melanaphis sacchari*) is a devastating pest of sorghum (*Sorghum bicolor*). Since it has been reported in 2013 in the United States as a serious pest of sorghum, efforts to control SCA have increased over the years. Sugarcane aphid overcome drastic climatic changes through colonizing sorghum in different developmental stages. Sugarcane aphid interactions with sorghum vary throughout the plant's development. Similar dynamics occur in the composition of epicuticular waxes of sorghum, where waxes differ throughout the growth process. However, the mechanisms behind the establishment of SCA and sorghum responses against them are not well understood. In this study, we used the Electrical Penetration Graph (EPG) technique to monitor aphid feeding behavior in two- and six-week-old sorghum 'BTx623' plants. No-choice bioassays were performed to assess the SCA population growth between two developmental stages. Differences in the abundance of the wax components in both developmental stages were also analyzed to determine whether SCA influence the wax composition of sorghum 'BTx623' plants. In summary, EPG data revealed that aphids spent more time in the sieve element phase of six-week-old plants, compared to two-week-old plants. Significant differences were found in the pathway phase and time spent to reach the first sieve element phase between the two-week and six-week-old plants. Interestingly, SCA population numbers were higher in two-week-old plants compared to

six-week-old plants. The abundance of the triterpenoids, α -amyrin and isoarborinone, increased after aphid infestation in six-week-old plants. Overall, our study provides insights into underlying the mechanisms involved in the SCA-sorghum interactions at different developmental stages.

Introduction

In recent years, the sugarcane aphid (SCA), *Melanaphis sacchari* (Zehntner, 1897), has become one of the most important pests in sorghum (Bowling et al., 2016). Efforts to mitigate their impact on sorghum have been of great magnitude in the United States. The success of this species is subject to various aspects of its biology (Brewer et al., 2017) and its ecology (Singh et al., 2004). The rapid growth of its populations, especially in warm environments and dry conditions (Neupane et al., 2019), its parthenogenetic reproduction and its effective dispersal strategy have ensured the success of this pest in almost all sorghum growing regions of the country (Brewer et al., 2017). Additionally, SCA directly attacks the plant, extracting all its nutrients through the sap-sucking mouth parts (Bowling et al., 2016), thereby weakening the plant. Each of these challenges has become a problem for the pest management of SCA in sorghum plants. Chemical treatment with insecticides is the most widely used management strategy to reduce SCA populations in sorghum (Szczepaniec 2018a). However, these strategies become unfeasible when the number of aphids/plants increases significantly. Additionally, the production of honeydew, a sticky digestive waste product of aphids composed largely of sugars, may greatly affect the harvesting process and cause reduction in crop yield (Szczepaniec 2018a).

Host plant resistance (HPR) is an alternative strategy to control SCA populations in the field (Bowling et al., 2016). Host plant resistance also allows us to understand more precisely the ecology and biology of the pest and permits the development of control mechanisms that are friendlier to the environment and more effective against the pest. One of the biggest challenges with SCA is its ability to colonize rapidly sorghum plants

and high dispersion through the overwintering range distribution by colonizing sorghum in vegetative stages in the southern U.S and in sorghum reproductive stages in the northern U.S (Kiani & Szczepaniec 2018). Thus, it is highly critical to understand the colonization timing of SCA for timely pest management (Szczepaniec 2018a).

Previously, it was shown that the changes in the population and the effects on sorghum plants in different developmental stages by the SCA are significant and represent an important factor for the knowledge of the biology and ecology of the pest (Kiani & Szczepaniec 2018). The SCA populations were almost 2.5 times larger in plants near reproductive stages of about six-week-old plants compared to plants in the vegetative stage of about two-week-old, suggesting that timely management of SCA is needed in the field (Szczepaniec 2018a; Szczepaniec 2018b). However, the underlying mechanisms and the ability of SCA to colonize sorghum plants at different developmental stages are poorly understood (Kiani & Szczepaniec 2018).

To determine and elucidate these mechanisms, the knowledge of the mechanisms involved in HPR provide endless methodologies and strategies to understand the mechanisms involved in these interactions. SCA is a sap-sucking insect that uses its stylets to reach plant sap for nutrient consumption (Zogli et al., 2020; Campbell & Dreyer 1985; Dreyer & Campbell 1987). This feeding behavior notably affects the vigor of the plant, and, on a large scale, it affects all biological and physiological processes causing severe injury, which may potentially result in plant death (Sharma et al., 1997).

The Electrical Penetration Graph (EPG) technique is used as one of the most powerful tools to monitor the insect feeding behavior (Tjallingii 1988). This technique provides detailed information associated with different feeding activities on the plant, including saliva

secretion and sap ingestion (Tjallingii 2006). Several studies have recently shown EPG as a good biological validation technique for the feeding behavior monitoring associated with plant resistance to piercing-sucking insects. This information is crucial to determine the numerous resistance mechanisms that plants develop under aphid infestation/herbivory (Grover et al., 2019; Tjallingii 2006).

Waxes play a fundamental role in several biological, physiological, and morphological processes of plants. The main function of waxes is to prevent water loss in drastic temperature conditions. In addition, being the first point of contact of the plant with the environment and its natural enemies, waxes also contribute to plant defense mechanisms, becoming a factor in determining the resistance or susceptibility of a plant (Shepherd et al., 1999a). The main components in waxes include n-alkanes, wax esters, free fatty alcohols, and free fatty acids as well as low levels of terpenoids, sterols, flavonoids, and phenolic substances (Busta et al., 2021). Additionally, this layer may also contain sugars, amino acids, and secondary plant substances such as glucosinolates, furanocoumarins and alkaloids (Eigenbrode & Espelie 1995; Wojcicka & Agnieszka 2015).

Waxes in plants are formed by two major components, the epicuticular wax layer and the intracuticular wax layer (Shepherd et al., 1999a). The epicuticular wax layer is the main component of cuticular waxes. Epicuticular waxes are formed by a complex mixture of long chain aliphatic, cyclic components, low levels of triterpenoids, sterols, flavonoids, and phenolic substances (Wojcicka & Agnieszka 2015, Kumar et al., 2017; Griffiths et al., 2000).

Epicuticular waxes are involved in a variety of processes in plants, which includes the production of important secondary metabolites related to plant defenses (Eigenbrode & Espelie 1995) and plant resistance upon herbivory (Sharma 2005; Shepherd et al., 1999a; Shepherd et al., 1999b). Previous studies have linked epicuticular waxes with antixenotic effects in plants against aphids (Verdugo et al., 2007). Waxes can alter the aphid adherence to the leaf (Shepherd et al., 2000; Pike et al., 2002; Smith 1999 & Gagic et al., 2016), and aphid movement (Bergman et al., 1991; Dixon et al., 1990). Moreover, Harris-Shultz et al. (2019) and Leszczynski et al. (2004) have found that the total number of aphids were lower in waxy plants.

Sugarcane aphid infestation in sorghum in the United States can be highly influenced by plant age and development and as mentioned above, in that sense, plant age is a crucial factor for the survival of this specie and its populations in sorghum. Additionally, studies carried out by Busta et al. (2021) showed that the composition and coverage of epicuticular waxes in sorghum plants differ greatly along plant development. Sorghum plants in vegetative stages (two-week-old) had higher wax coverage but lower abundance of wax components compared to plants close to reproductive stages (six-week-old). In contrast, six-week-old sorghum plants had lower wax coverage, but higher abundance of wax components compared to two-week-old plants (Busta et al., 2021). These marked differences in epicuticular waxes may interfere in SCA-sorghum interactions at different stages of development.

Because pest management strategies against SCA in sorghum are still limited, improving our knowledge of SCA feeding, survival and behavior is essential to better understand the performance of SCA in sorghum. In addition, the analysis of factors that can influence the insect-plant interactions, such as the role of epicuticular waxes in these interactions, is highly important for implementing sustainable pest management strategies. Thus, in this study, we monitor the SCA feeding behavior through the EPG technique in two different developmental stages of sorghum wild-type (BTx623) plants and evaluate the role of epicuticular wax in two different developmental stages of sorghum after SCA infestation.

Materials and methods

Plant material

The sorghum (*Sorghum bicolor*) BTx623 genotype used in this study is the considered as the reference line for sorghum. Two-week-old and six-week-old sorghum plants were grown in the University of Nebraska-Lincoln (UNL) greenhouse with a 16-h-light/8-h-dark photoperiod, 25 °C, and 50–60% relative humidity. Seeds were sown in soil mixed with vermiculite and perlite (PRO-MIX BXBIOFUNGICIDE + MYCORRHIZAE, Premier Tech Horticulture Ltd., Canada) in Cone-Tainers (Ray Leach SC10; Stuewe & Sons, Inc., Tangent, OR). Experiments with two-week-old and six-week-old plants were initiated as the same time when plants reached the appropriate age.

Insect colony

The SCA colony was maintained as previously described (Grover et al., 2020) on the susceptible BCK60 sorghum genotype in a growth chamber with 16-h-light/8-h-dark

photoperiod, $140 \mu\text{E m}^{-2} \text{s}^{-1}$ light quality, $23 \text{ }^\circ\text{C}$, and 50–60% relative humidity. The BCK60 sorghum plants for aphid rearing were grown in the greenhouse until it reached 7-leaf stage. New plants were substituted with old, deteriorated plants in the growth chamber, whenever necessary. For all the experiments, adult aphids were used and transferred to experimental plants with a fine-bristled paintbrush.

Aphid No-Choice assay

Multiple no-choice assay was conducted with SCA in two-week-old and six-week-old potted BTx623 plants. A Completely Randomized Design (CRD) was utilized to determine aphid growth and survival in both developmental stages. For this experiment, sixteen (16) two-week-old and six-week-old plants were randomly organized and infested with 5 adult apterous aphids. Adults were placed in the adaxial side of the leave in two-week-old plants and at the top of the whorl leaf in six-week-old plants. Succeeding the infestation of two-week-old plants, individual plants were caged with tubular clear plastic, and ventilated with organdy fabric on the top and sides. Plants of six-week-old were caged using cloth bags and a woody stick was used to guarantee the plant support. The total number of aphids including adults and nymphs were counted after 10 days of infestation on each developmental stage.

Aphid feeding behavior analysis

EPG recording

Two-week- and six-week-old BTx623 plants were used for monitoring the SCA feeding behavior. Adult apterous SCA were individually placed on each plant at the center of the

adaxial leaf lamina. The experimental procedures and aphid wiring were conducted as described previously (Tetreault et al., 2019). Briefly, aphids were starved for 1 h in a plastic petri dish prior to EPG recording. Using a stereoscope, a brass nail with a gold wire attached (insect electrode) was glued to dorsum of aphids using a silver conductive glue obtained by mixing 4 mL water with a single drop of Triton X-100, 4 mg water-soluble glue (Scotch clear paper glue, non-toxic; 3 M, St. Paul, MN, USA), and 4 g silver flake (99.95%, size, 8–10 μm , Inframat Advanced Materials, Manchester, CT, USA). To complete the basic circuit, a plant electrode (stiff copper wire) was introduced into the soil surrounding the potted plant. For measurements, a Giga-8 EPG (EPG Systems, Wageningen, The Netherlands) with a $10^9 \Omega$ resistance amplifier was connected to each of the electrodes and an adjustable plant voltage were used for measuring feeding behavior of SCA on sorghum plants. Experiments using the EPG technique were conducted at laboratory conditions at 22–24 °C and 40–45% RH under continuous light conditions. Four plants with same age were placed at a time randomly in a Faraday's cage for the recordings. All EPG recordings were started between 8 am - 10 am local time (U.S. Central Standard Time). Overall, 14 replications were used for each developmental stage of recordings for 8 h. EPG acquisition software (*Stylet+*, EPG Systems, Wageningen, The Netherlands) was used to record EPG waveforms.

Feeding behavior parameters

Four categorized EPG waveform phases/patterns were considered in this study: pathway phase, which correspond to the penetration and removal of aphid stylets intercellularly; xylem phase, corresponding to water ingestion; sieve element phase (phloem phase),

indicating ingestion of phloem sap (in this phase, the aphid salivation and passive ingestion, E1 and E2, respectively, were also monitored (Grover et al., 2020)) and the non-probing phase. The non-probing phase shows the interval of relatively no stylet movement or not probing into the plant. For more detailed results, additional parameters were measured: the number of potential drops which correspond to intracellular punctures, time to first probe, which is the time difference between the starting of recording and the first stylet insertion into plant, and finally, the time to first sieve elements once the recording was initiated.

Wax composition analysis

For wax composition analysis, plants were infested with aphids as described above for the no-choice assays and leaf tissues were collected after 10 days of SCA feeding. SCA uninfested plants were used as the control plants for both developmental stages (two-week-old and six-week-old). For leaf sample collection, we carefully placed the second most developed leaf of the plant in a hole puncher of approximately 3 cm² in area, without manipulating or contaminating the collection area. Once the leaf is located in the hole puncher near to the tip of the leaf, we punched out one leaf disc. A total of three leaf punches were considered a replication in each of the treatments. The leaf discs were placed directly into a vial of polypropylene cap and polyethylene liner (20 mL 28 x 61 mm (with Cap)) (Busta et al., 2021) and capped for storage/transport. The protocol for the analysis of the epicuticular waxes present in each of the samples in the four different treatments was as described previously (Busta et al., 2021).

Statistical analyses

EPG data was analyzed using a non-parametric Kruskal–Wallis test in nine different feeding phases/patterns for each developmental stage. Considering the non-normality distribution of the data, the PROC NPAR1WAY procedure was used. Multiple comparisons of different treatments between the means were performed using SAS. For the no-choice assay and wax composition data analysis, comparisons were performed using a t-test with normal LSD ($\alpha = 0.05$). Values presented are least square means and standard error.

Results

SCA survival and reproduction was higher on two-week-old plants

SCA survival and reproduction was higher in two-week-old plants after 10 days of infestation compared to six-week-old plants (Fig 2.1). SCA aphid population was almost twice the size (average mean population = 749) in two-week-old plants compared to six-week-old plants (average mean population = 421.3) (Fig 2.1).

SCA spent more time in phloem feeding on six-week-old plants

A Representative EPG waveform of each of the developmental stage is shown in Fig 2.2. Our results demonstrate that the aphids spent longer time in the sieve elements phase in six-week-old plants compared to two-week-old plants (Fig 2.3). On the other hand, aphids spent less time in the pathway phase in six-week-old plants compared to two-week-old plants. No significant differences were found in the xylem and non-probing phases between the different developmental stages (Fig 2.3).

SCA took less time to reach phloem phase on six-week-old plants

Aphids started feeding faster in six-week-old plants compared to two-week-old plants in about half of the time, suggesting that the SCA preferred to feed on sorghum plants in advanced stage of development compared to young sorghum plants (Fig 2.4A). No significant differences were found in the time to first probe (Fig 2.4A) and number of potential drops (Fig 2.4B) between both developmental stages. Aphids started probing on six-week-old and two-week-old sorghum BTx623 plants in about at the same time. Similarly, we observed comparable number of probing attempts made by SCA on both developmental stages of sorghum.

SCA spent more time in passive ingestion of phloem sap on six-week-old sorghum plants

Considering the differences in the phloem phase between both developmental stages, we also analyzed the phloem phase in detail. The analysis was performed for the E1 phase, or salivary phase, and the E2, or passive ingestion phase. However, based on obtained waveforms in our analysis we also included a third phase that consists of combination of patterns found when the aphids are salivating before the passive ingestion phase. In this context, the alternance between E1 and E2 phase is commonly denominated as the transition phase (Will et al., 2007). Our results confirm that the aphids spent more time feeding in the passive ingestion phase of six-week-old plants (Fig 2.5). Aphids spent ~1 more hour during the passive ingestion in six-week-old plants compared to two-week-old plants. Although there were not significantly differences in E1 or salivation phase

between both developmental stages, we observed a longer salivation period in six-week-old plants (Fig 2.5). Finally, no significant differences were found in transition phase in both developmental stages.

Abundance of the triterpenoids α -amyrin and isoarborinone increased after aphid infestation in six-week-old plants

Here, we quantified the relative abundance ($\mu\text{g}/\text{cm}^2$) of each of the wax components present in the leaf sample (3 cm^2) between two-week-old plants and six-week-old plant before (control) and after SCA infestation (Fig 2.6). In total, 15 compounds were detected from the samples collected for each developmental stage. The compounds with the highest abundance were those derived from alcohols and triterpenoids in both two-week-old and six-week-old plants; however, most of these compounds detected in the analysis were present in greater proportion in the six-week-old plants compared to the two-week-old plants. Although no major differences were found regarding the abundance of components between the developmental stages, differences were found in the abundance values after SCA infestation in both the two-week-old plants and the six-week-old plants. Significant increases were evidenced in the abundance values of α -amyrin and isoarborinone compounds, both compounds belong to the triterpenoid family, after SCA infestation in six-week-old plants. However, no significant differences were observed in the abundance of these two compounds in two-week-old plants after SCA infestation.

Discussion

The present study provides detailed information on the SCA feeding behavior in sorghum BTx623 plants in two different developmental stages, in the vegetative stages (two-week-

old) and stages close to the reproductive period (six-week-old). Analysis of epicuticular waxes show changes in the abundance of compounds α -amyrin and isoarborinone after SCA infestation in two-week-old plants. In addition, the no-choice assay revealed that the SCA proliferation was higher in two-week-old plants compared to six-week-old plants.

Interestingly, our results demonstrate that the SCA proliferation and aphid count numbers were higher in plants during the vegetative stage. This result is in contrast to a previous study, where they reported higher number of aphids in reproductive stages of sorghum plants (Kiani & Szczepaniec 2018; Szczepaniec 2018a). Difference in sorghum genotypes used in their study and our study could be one of the differences that may contribute to this disparity. Alternatively, the genotypes used in their study may have more sugar-rich phloem sap, which is the major nutrient source for aphids, compared to BTx623 genotype used in our study. Further experiments are needed to determine if the different results are due to differences in sugar concentrations between this study and that of Kiani & Szczepaniec (2018).

Our EPG data revealed that SCA established a prolonged feeding duration on the phloem sap of the six-week-old plants, and additionally spent less time in the pathway phase in search for a possible spot to get the nutrients from the plants. To complement this data, SCA spent longer time in the passive ingestion phase in two-week-old plants, a phase in which nutrients are obtained by the SCA from the plants (Fartek et al., 2012). These results suggest that the SCA was able to locate the necessary nutrients relatively faster in six-week-old plants compared to two-week-old plants.

Epicuticular waxes are involved in many plants defense processes, The components and the crystal structures present in the waxes (White & Eigenbrode 2000) may be related to the facilitation of adherence in plants (Friedemann et al., 2015). In our study, the compounds α -amyrin and isoarborinone increased with the presence of SCA in six-week-old plants, both compounds belong to the family of triterpenoids. Triterpenoids are widely known and have been reported in several studies as a major factor that modulates insect-plant interactions (González-Coloma et al., 2011). In several studies (Tamura et al., 2004; Robertson et al., 1991; Shepherd et al., 1999a; Eigenbrode & Espelie 1995) it has been shown α -amyrin is a feeding stimulant in insects. It is highly likely that the SCA's preference to feed and settle on six-week-old plants may be due to increased triterpenoids derivatives, which may be acting as feeding stimulants (Tamura et al., 2004).

This study describes the possible defensive responses of sorghum to SCA infestation at multiple developmental stages. Although the information related to this type of interaction is widely studied, little is known about the underlying mechanisms that contribute to defense in different developmental stages of sorghum. As previously mentioned, it is critical to understand the responses of plants that they initially trigger with respect to the first point of contact between themselves and the environment. The knowledge and description of mechanisms that manage to demonstrate the possible physiological and morphological changes in plants upon herbivory can broaden the range of possibilities for pest management through strategies and mechanisms based on HPR. Therefore, this study is important since it denotes basic mechanisms of response in plants

to SCA infestation in early stages of development versus stages close to the reproductive period, which are the two significant stages of plant development.

Acknowledgments

I would like to thank Dr. Lucas Busta for the contributions in sharing valuable information related with his research in sorghum wax data and also helping me in the analysis of waxes.

References

- Bergman, D. K., Dillwith, J. W., Zarrabi, A. A., Caddel, J. L., & Berberet, R. C.** (1991). Epicuticular lipids of alfalfa relative to its susceptibility to spotted alfalfa aphids (Homoptera: Aphididae). *Environmental entomology*, 20(3), 781-785.
- Bowling, R. D., Brewer, M. J., Kerns, D. L., Gordy, J., Seiter, N., Elliott, N. E., Buntin, G.D., Way, M.O., Royer, T.A., Biles, S., & Maxson, E.** (2016). Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. *Journal of Integrated Pest Management*, 7(1), 12; 1-13.
- Brewer, M. J., Gordy, J. W., Kerns, D. L., Woolley, J. B., Rooney, W. L., & Bowling, R. D.** (2017). Sugarcane aphid population growth, plant injury, and natural enemies on selected grain sorghum hybrids in Texas and Louisiana. *Journal of economic entomology*, 110(5), 2109-2118.
- Busta, L., Schmitz, E., Kosma, D. K., Schnable, J. C., & Cahoon, E. B.** (2021). A co-opted steroid synthesis gene, maintained in sorghum but not maize, is associated with a divergence in leaf wax chemistry. *Proceedings of the National Academy of Sciences*, 118(12).
- Campbell, B. C., & Dreyer, D. L.** (1985). Host-plant resistance of sorghum: differential hydrolysis of sorghum pectic substances by polysaccharases of greenbug biotypes (*Schizaphis graminum*, Homoptera: Aphididae). *Archives of Insect Biochemistry and Physiology*, 2(2), 203-215.
- Dixon, A. F. G., Croghan, P. C., & Gowing, R. P.** (1990). The mechanism by which aphids adhere to smooth surfaces. *Journal of Experimental Biology*, 152(1), 243-253.
- Dreyer, D. L., & Campbell, B. C.** (1987). Chemical basis of host-plant resistance to aphids. *Plant, Cell & Environment*, 10(5), 353-361.
- Eigenbrode, S. D., & Espelie, K. E.** (1995). Effects of plant epicuticular lipids on insect herbivores. *Annual review of entomology*, 40(1), 171-194.
- Fartek, B., Nibouche, S., Turpin, P., Costet, L., & Reynaud, B.** (2012). Resistance to *Melanaphis sacchari* in the sugarcane cultivar R 365. *Entomologia Experimentalis et Applicata*, 144(3), 270-278.
- Friedemann, K., Kunert, G., Gorb, E., Gorb, S. N., & Beutel, R. G.** (2015). Attachment forces of pea aphids (*Acyrtosiphon pisum*) on different legume species. *Ecological Entomology*, 40(6), 732-740.
- Gagic, V., Petrović-Obradović, O., Fründ, J., Kavallieratos, N. G., Athanassiou, C. G., Starý, P., & Tomanović, Ž.** (2016). The effects of aphid traits on parasitoid host use and specialist advantage. *PloS one*, 11(6), e0157674.
- Gerik, T., Bean, B. W., & Vanderlip, R.** (2003). Sorghum growth and development. Texas FARMER Collection; B6107; 7-03.

- Gonzales, W. L., Ramirez, C. C., Olea, N., & Niemeyer, H. M.** (2002). Host plant changes produced by the aphid *Sipha flava*: consequences for aphid feeding behaviour and growth. *Entomologia Experimentalis et Applicata*, 103(2), 107-113.
- González-Coloma, A., López-Balboa, C., Santana, O., Reina, M., & Fraga, B. M.** (2011). Triterpene-based plant defenses. *Phytochemistry Reviews*, 10(2), 245-260.
- Griffiths, D. W., Robertson, G. W., Shepherd, T., Birch, A. N. E., Gordon, S. C., & Woodford, J. A. T.** (2000). A comparison of the composition of epicuticular wax from red raspberry (*Rubus idaeus* L.) and hawthorn (*Crataegus monogyna* Jacq.) flowers. *Phytochemistry*, 55(2), 111-116.
- Grover, S., Agpawa, E., Sarath, G., Sattler, S. E., & Louis, J.** (2020). Interplay of phytohormones facilitate sorghum tolerance to aphids. *Plant Molecular Biology*, 1-12.
- Grover, S., Wojahn, B., Varsani, S., Sattler, S. E., & Louis, J.** (2019). Resistance to greenbugs in the sorghum nested association mapping population. *Arthropod-Plant Interactions*, 13(2), 261-269.
- Grover, S., Varsani, S., Kolomiets, M. V., & Louis, J.** (2020). Maize defense elicitor, 12-oxo-phytodienoic acid, prolongs aphid salivation. *Communicative & Integrative Biology*, 13(1), 63-66.
- Harris-Shultz, K., Punnuri, S., Knoll, J. E., Ni, X., & Wang, H.** (2020). The sorghum epicuticular wax locus *Bloomless2* reduces plant damage in P898012 caused by the sugarcane aphid. *Agrosystems, Geosciences & Environment*, 3(1), e20008.
- He, J., Chen, F., Chen, S., Lv, G., Deng, Y., Fang, W., Liu, Z., Guan, Z., & He, C.** (2011). Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation. *Journal of plant physiology*, 168(7), 687-693.
- Khare, S., Singh, N. B., Singh, A., Hussain, I., Niharika, K., Yadav, V., Bano, C., Yadav, R.K., & Amist, N.** (2020). Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. *Journal of Plant Biology*, 63(3), 203-216.
- Khattab, H.** (2007). The defense mechanism of cabbage plant against phloem-sucking aphid (*Brevicoryne brassicae* L.). *Australian Journal of Basic and Applied Sciences*, 1(1), 56-62.
- Kiani, M., & Szczepaniec, A.** (2018). Effects of sugarcane aphid herbivory on transcriptional responses of resistant and susceptible sorghum. *BMC genomics*, 19(1), 1-18.
- Knoll, J. E., Uchimiya, M., & Harris-Shultz, K.** (2021). Juice chemical properties of 24 sorghum cultivars under varying levels of sugarcane aphid (*Melanaphis sacchari*) infestation. *Arthropod-Plant Interactions*, 15(5), 707-719.

- Kumar, S., Singh, Y.P., Singh, S.P., & Singh, R.** (2017). Physical and biochemical aspects of host plant resistance to mustard aphid, *Lipaphis erysimi* (Kaltenbach) in rapeseed-mustard. *Arthropod-Plant Interactions* 11, 551–559.
- Leszczynski, Bogumil & Wójcicka, Agnieszka & Matok, Henryk & Urbańska, Anna & Krzyżanowski, Robert.** (2004). Mechanisms of cereal resistance towards aphids. *Aphids and Other Hemipterous Insects*. 10. 75-85.
- Machado, R. A., Arce, C. C., Ferrieri, A. P., Baldwin, I. T., & Erb, M.** (2015). Jasmonate-dependent depletion of soluble sugars compromises plant resistance to *Manduca sexta*. *New Phytologist*, 207(1), 91-105.
- Neupane, S. B., Kerns, D. L., & Szczepaniec, A.** (2020). The impact of sorghum growth stage and resistance on life history of sugarcane aphids (Hemiptera: Aphididae). *Journal of economic entomology*, 113(2), 787-792.
- Ogbonnaya, C. A.** (1985). Influence of biotype E greenbugs on biochemical properties of sorghum (Doctoral dissertation, The University of Nebraska-Lincoln).
- Pike, N., Richard, D., Foster, W., & Mahadevan, L.** (2002). How aphids lose their marbles. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1497), 1211-1215.
- Smith, R. G.** (1999). Wax glands, wax production, and the functional significance of wax use in three aphid species (Homoptera: Aphididae). *Journal of Natural History*, 33(4), 513-530.
- Robertson, G. W., Griffiths, D. W., Birch, A. N. E., Jones, A. T., McNicol, J. W., & HALL, J. E.** (1991). Further evidence that resistance in raspberry to the virus vector aphid, *Amphorophora idaei*, is related to the chemical composition of the leaf surface. *Annals of applied biology*, 119(3), 443-449.
- Roozeboom, K. L., & Prasad, P. V.** (2016). Sorghum growth and development. *Sorghum: State of art and future perspective*, 1-18.
- Sharma, H. C., & Dhillon, M. K.** (2005). Reaction of different sorghum genotypes to infestation by the sugarcane aphid, *Melanaphis sacchari* Zehntner. *Indian Journal of Entomology*, 67(4), 291-296.
- Sharma, H. C., Nwanze, K. F., & Subramanian, V.** (1997). Mechanisms of resistance to insects and their usefulness in sorghum improvement. *Plant resistance to insects in sorghum*, 81-100.
- Shepherd, T., Robertson, G. W., Griffiths, D. W., & Birch, A. N. E.** (2000). Plants and aphids: the chemical ecology of infestation. *Scottish Crop Research Institute Annual Report 1999, 2000*, 122-124.

- Shepherd, T., Robertson, G. W., Griffiths, D. W., & Birch, A. N. E.** (1999a). Epicuticular wax composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeus* L.). *Phytochemistry*, 52(7), 1239-1254.
- Shepherd, T., Robertson, G. W., Griffiths, D. W., & Birch, A. N. E.** (1999b). Epicuticular wax ester and triacylglycerol composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeus* L.). *Phytochemistry*, 52(7), 1255-1267.
- Singh, B. U., Padmaja, P. G., & Seetharama, N.** (2004). Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner)(Homoptera: Aphididae), in sorghum: a review. *Crop Protection*, 23(9), 739-755.
- Szczepaniec, A.** (2018a). Interactive effects of crop variety, insecticide seed treatment, and planting date on population dynamics of sugarcane aphid (*Melanaphis sacchari*) and their predators in late-colonized sorghum. *Crop protection*, 109, 72-79.
- Szczepaniec, A.** (2018b). Assessment of a density-based action threshold for suppression of sugarcane aphids, (Hemiptera: Aphididae), in the Southern High Plains. *Journal of economic entomology*, 111(5), 2201-2207.
- Tamura, Y., Hattori, M., Konno, K., Kono, Y., Honda, H., Ono, H., & Yoshida, M.** (2004). Triterpenoid and caffeic acid derivatives in the leaves of ragweed, *Ambrosia artemisiifolia* L.(Asterales: Asteraceae), as feeding stimulants of *Ophraella communa* LeSage (Coleoptera: Chrysomelidae). *Chemoecology*, 14(2), 113-118.
- Tetreault, H. M., Grover, S., Scully, E. D., Gries, T., Palmer, N. A., Sarath, G., Louis, J., & Sattler, S. E.** (2019). Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphid (*Melanaphis sacchari*). *Frontiers in plant science*, 10, 145.
- Tjallingii, W. F.** (1988). Electrical recording of stylet penetration activities. In *Aphids, their biology, natural enemies, and control*. Elsevier Science Publishers, (pp. 95-108).
- Tjallingii, W. F.** (2006). Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of experimental botany*, 57(4), 739-745.
- Verdugo, J. A., Astudillo, L. A., & Ramírez, C. C.** (2007). Effect of epicuticular waxes of poplar hybrids on the aphid *Chaitophorus leucomelas* (Hemiptera: Aphididae). *Journal of applied entomology*, 131(7), 486-492.
- Villanueva, R. T., & Sekula, D.** (2014). A new pest of sorghum: The sugarcane aphid. In 20th Annual Rio Grande Valley Cotton & Grain Pre-Plant Conference. counties. AgriLife.
- Walker G.P.** (2000). Beginner's guide to electronic monitoring, in *Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior*, ed. by Walker GP and Backus EA. Entomological Society of America, Lanham, MD, pp. 14-40.

- White, C., & Eigenbrode, S. D.** (2000). Effects of surface wax variation in *Pisum sativum* on herbivorous and entomophagous insects in the field. *Environmental Entomology*, 29(4), 773-780.
- Will, T., Tjallingii, W. F., Thönnessen, A., & van Bel, A. J.** (2007). Molecular sabotage of plant defense by aphid saliva. *Proceedings of the National Academy of Sciences*, 104(25), 10536-10541.
- Wójcicka, A.** (2015). Surface waxes as a plant defense barrier towards grain aphid. *Acta Biologica Cracoviensia s. Botanica*, 57(1).
- Zogli, P., Pingault, L., Grover, S., & Louis, J.** (2020). Ento (o) mics: the intersection of 'omic' approaches to decipher plant defense against sap-sucking insect pests. *Current Opinion in Plant Biology*, 56, 153-161.

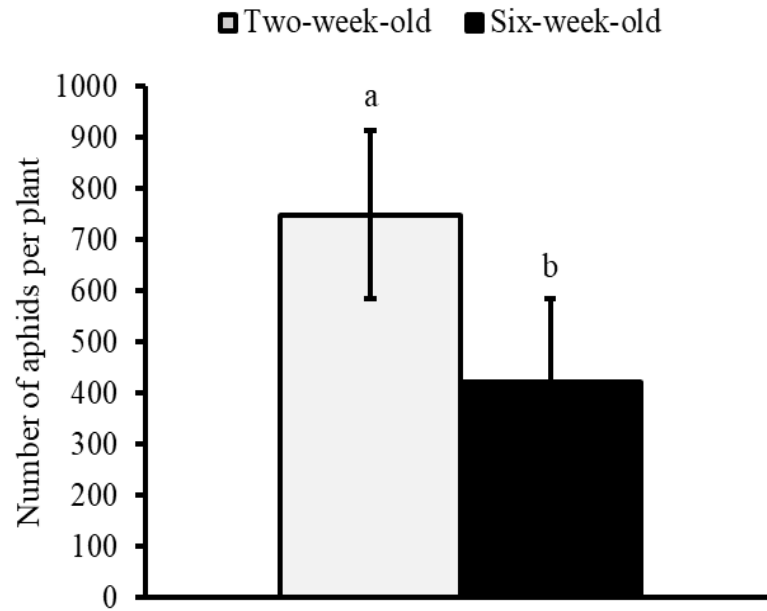


Figure 2.1

Total number of SCA adults and nymphs per plant after 10 days of infestation in two-week-old and six-week-old BTx623 plants are shown. $n = 16$ for each developmental stage. Bars denote the SCA mean pooled number of adults and nymphs per plant in both developmental stages. Different letter above the bars means significant statistical differences ($P < 0.05$). Error bars represent \pm SEM.

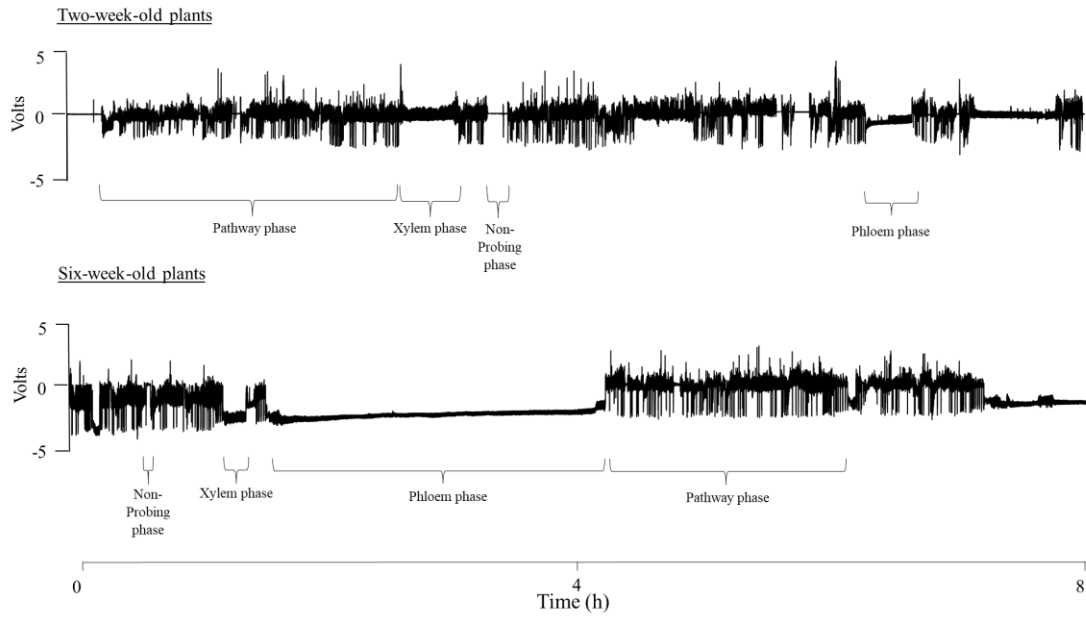


Figure 2.2

EPG waveforms representing the SCA aphid feeding behavior in two-week-old (top panel) and six-week-old (bottom panel) in sorghum BTx623 plants for 8 h.

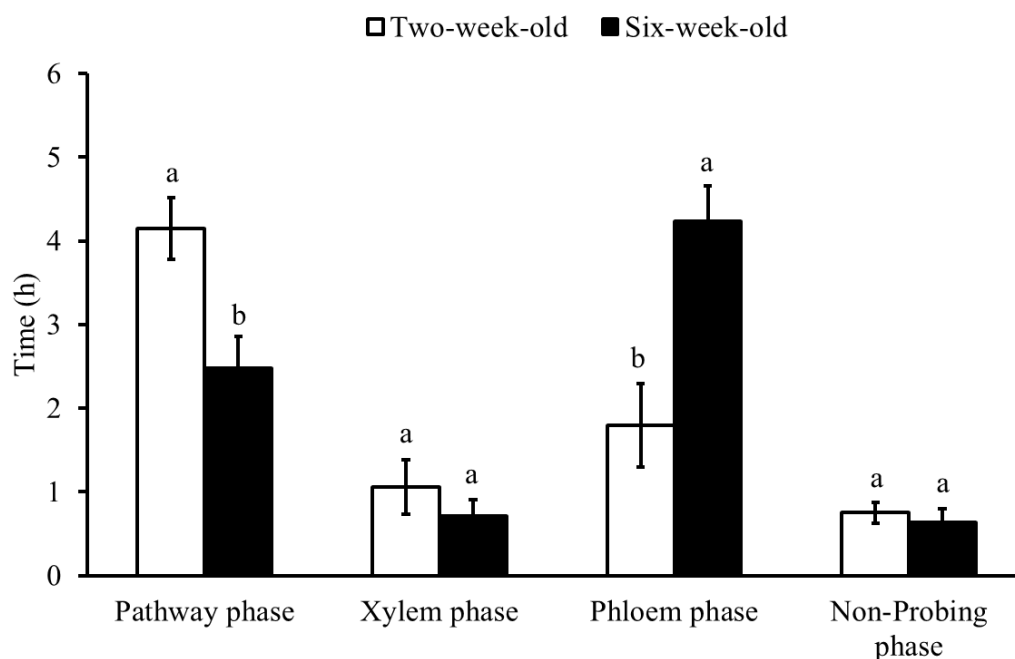


Figure 2.3

SCA feeding behavior parameters in six-week-old and two-week-old sorghum BTx623 plants for 8 h of feeding duration. The total time spent by SCA for different feeding behavior parameters in each of the developmental stages is shown. $n = 14$. Bars denote the mean values obtained for six-week-old and two-week-old sorghum BTx623 plants. Bars with different letters are significantly different from one another (Kruskal-Wallis test and multiple comparisons; $P < 0.05$). Error bars represent \pm SEM.

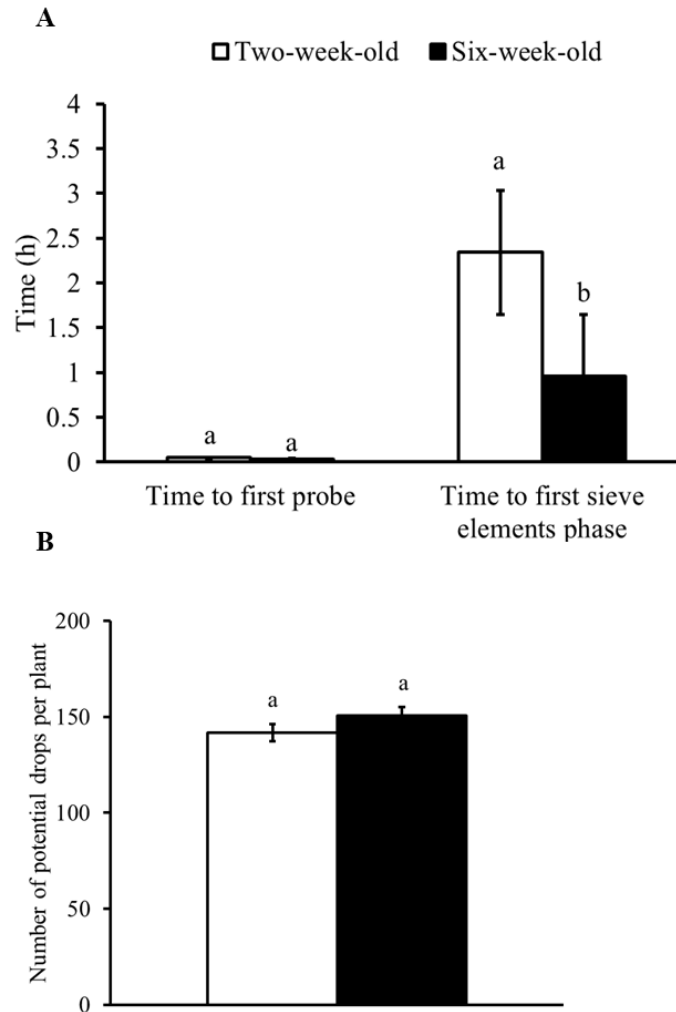


Figure 2.4

A. Time taken by SCA to first probe and time to first sieve element in six-week-old and two-week-old sorghum BTx623 plants; **B.** Mean number of potential drops in six-week-old and two-week-old sorghum BTx623 plants in 8 h duration of SCA feeding. $n = 14$. Bars denote the mean values obtained for six-week-old and two-week-old Sorghum BTx623 plants. Bars with different letters are significantly different from one another (Kruskal-Wallis test and multiple comparisons; $P < 0.05$). Error bars represent \pm SEM.

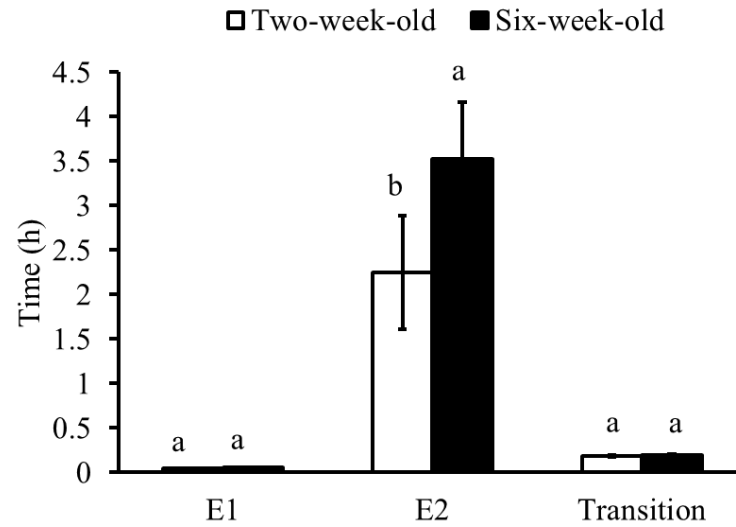
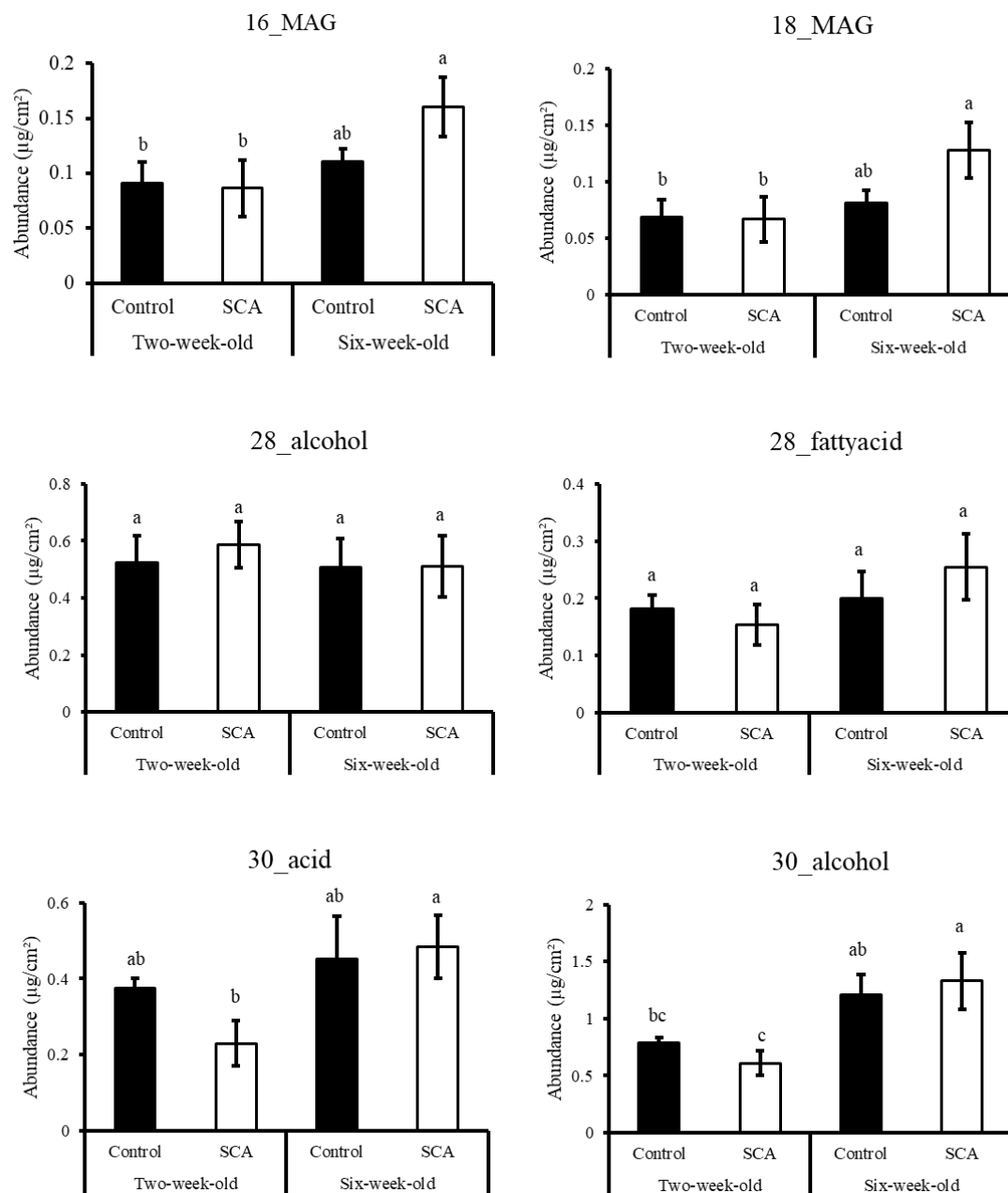
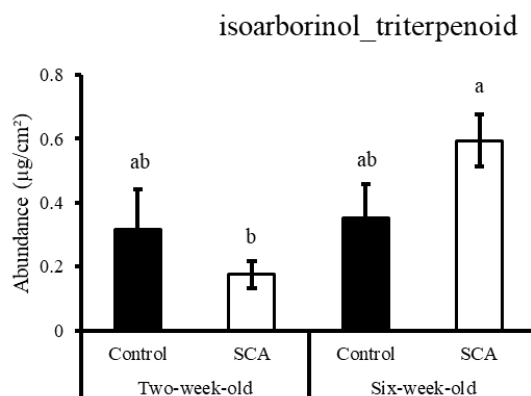
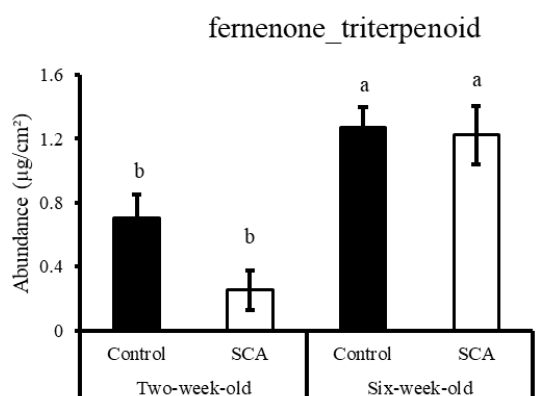
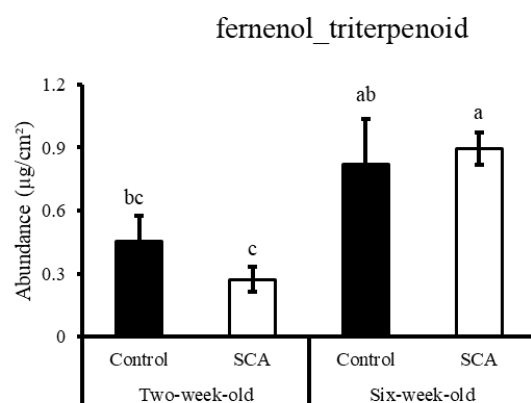
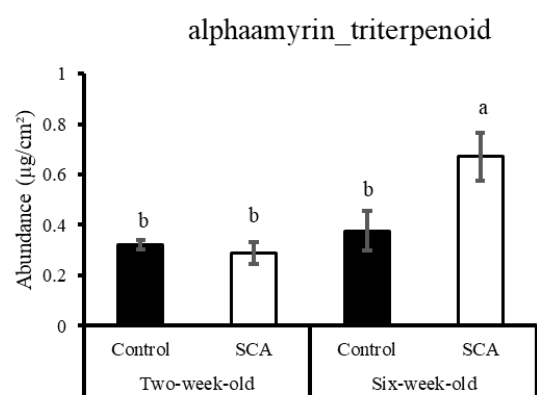
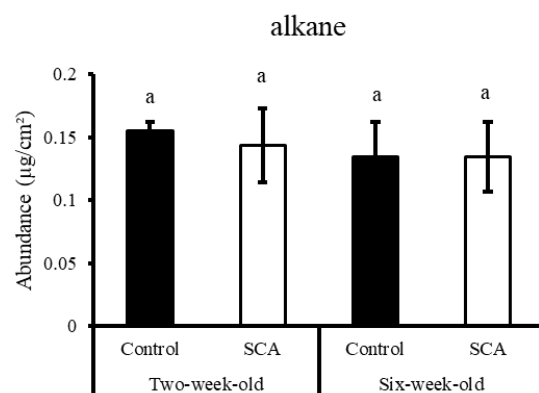
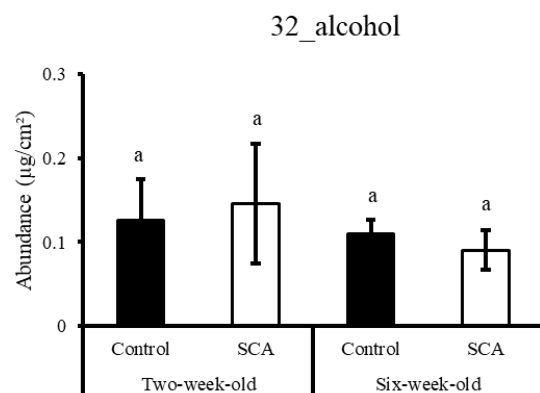


Figure 2.5

Detailed sieve element phase analysis. Salivary (E1), passive ingestion (E2) and transition phases in six-week-old and two-week-old sorghum BTx623 plants in 8 h duration of SCA feeding. $n = 14$. Bars denote the mean values obtained for six-week-old and two-week-old sorghum BTx623 plants. Bars with different letters are significantly different from one another (Kruskal-Wallis test and multiple comparisons; $P < 0.05$). Error bars represent \pm SEM.





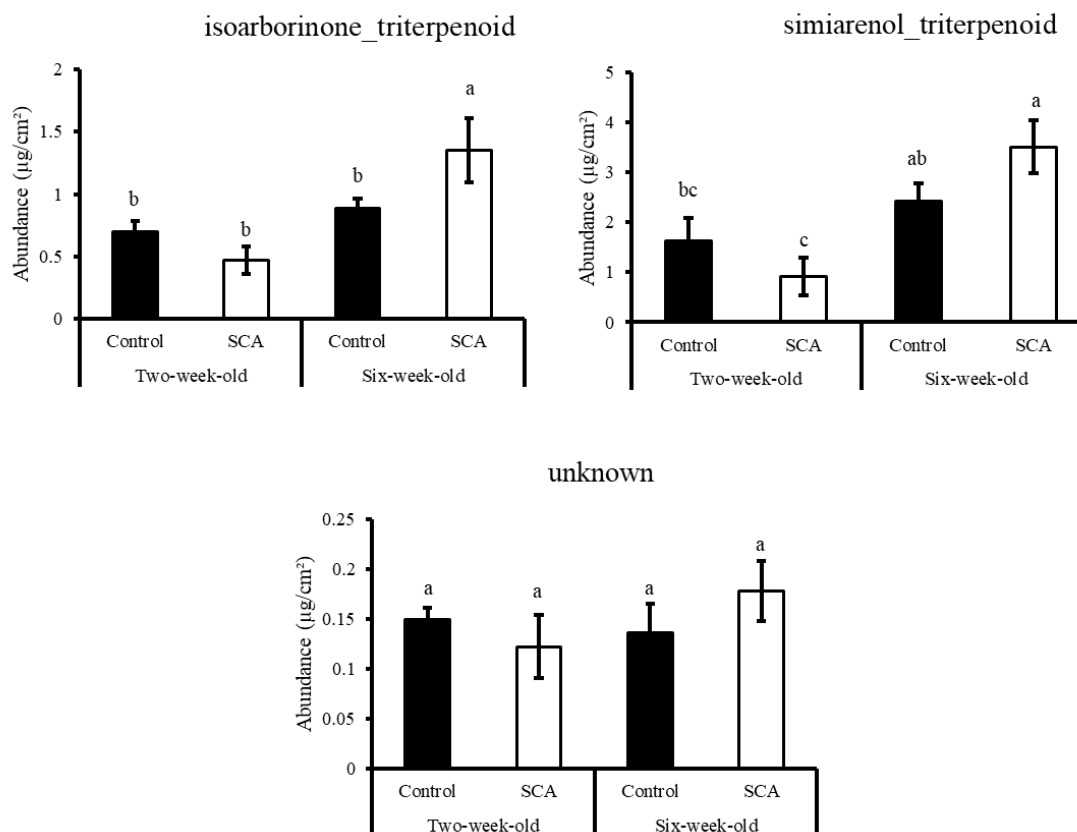


Figure 2.6

Abundance ($\mu\text{g}/\text{cm}^2$) of each of the wax components present in the leaf sample (3 cm^2) between two-week-old plants and six-week-old plants before (control) and 10 days after SCA infestation; $n = 6$. Bars with different letters are significantly different from one another ($\alpha = 0.05$). Error bars represent \pm SEM.

CHAPTER 3

Role of epicuticular waxes in sorghum resistance to sugarcane aphids (SCA)

(*Melanaphis sacchari*)

Abstract

Sugarcane aphid (SCA: *Melanaphis sacchari*) is a relatively new and devastating pest of sorghum (*Sorghum bicolor*) in the United States. Although mechanisms that explain these interactions have been described over the years, the establishment and effectiveness of SCA on sorghum is still poorly understood. The epicuticular wax (EW) layer constitutes the first point of contact between the insect-plant interactions. It is known that the composition of the EW can influence many of the mechanisms of plant responses in the presence of aphids; however, the performance of SCA in plant genotypes with and without wax components is still unknown. With the aim to better understand SCA performance and settling on sorghum wax mutants and wild-type plants, we performed choice and no-choice assays between the sorghum wild-type and bloomless plants, which is a wax mutant. Additionally, we used the EPG technique to monitor SCA feeding behavior in these two different lines. We also quantified the wax abundance to correlate wax composition in two-week-old plants and aphid performance and settling in both genotypes. The total amount of 16-monoacylglycerols and 32C-alcohols was higher in bloomless plants compared to the wild-type plants. SCA reproduction did not alter between the wild-type and bloomless plants. The choice assay experiment revealed that the SCA preferred to settle on bloomless plants compared to wild-type plants. EPG analysis demonstrated that the SCA spent more time feeding on the xylem sap of the

bloomless plants compared to the wild-type plants. Overall, our study provides insights on the role of epicuticular waxes in SCA settling and performance in sorghum plants.

Introduction

There are many characteristics that make sorghum (*Sorghum bicolor*) one of the most used and cultivated cereals in the world (Venkateswaran et al., 2019). Sorghum is used for animal and human consumption, biomass production to generate biofuels (Stamenković et al., 2020), production of syrup (McGinnis & Painter 2020), production of nutrients with high dietary content, and some of their compounds are implemented in the pharmaceutical industry (Espitia-Hernández et al., 2020).

The sugarcane aphid (SCA) (*Melanaphis sacchari*) is a key pest of sorghum around the world (Singh et al., 2004). In 2013, it was described as a pest on sorghum in the United States (Villanueva et al., 2014). To date, SCA populations have rapidly increased and is now being reported in 29 states (EDDMapS., 2022). Similar to other aphids, SCA is a piercing-sucking insect (Singh et al., 2004) that ingest the plant nutrients by penetrating the plant leaf and stalk tissues using a straw-shaped stylet (Walker 2000). Direct loss of plant nutrients from SCA feeding can cause stress, loss of vigor, changes in pigmentation and plant decline. Additionally, SCA honeydew production reduces the photosynthetic capacity of the plant and can ultimately lead to plant death (Singh et al., 2004)

The Epicuticular Waxes (EW) constitute the first point of interaction between the aphid and the plant (Eigenbrode & Espelie 1995). This structure primarily protects sorghum from desiccation, making sorghum an ideal crop for dry environments. These structures are mainly composed of mixtures of aliphatic acid and cyclic components such as fatty acids, hydrocarbons, alcohols, aldehydes, ketones, β -iketones and esters and low levels of

triterpenoids, sterols, flavonoids, and phenolic substances (Wójcicka 2016). However, EW have been found to play an important role in insect-plant interactions (Eigenbrode & Espelie 1995). This layer may also contain sugars, amino acids, and secondary plant substances such as glucosinolates, furanocoumarins and alkaloids (Eigenbrode & Espelie 1995), which are found to contribute to plant defenses upon herbivory (Harborne 1991). Taken together, the chemical composition, structure, and nature of epicuticular waxes may influence interactions between aphids and sorghum plants.

The EW in sorghum represents an important component in its survival. Numerous studies have reported that the presence or lack of EW influence the performance of sorghum itself (Punnuri et al., 2017a) and the sorghum-insect interactions (Punnuri & Huang 2017). Ayyangar et al. (1937) made the first report of bloomless plants, where these sorghum plants completely lacked EW compared to the respective wild-type (bloom phenotype) plants. Most studies with EW have focused on understanding how their chemical composition affects aphid performance. However, little is known about the aphid performance in plants with the bloomless phenotype versus plants with the bloom phenotype (Wójcicka 2016).

Previous studies have shown that the absence of EW conferred resistance to greenbug (*Schizaphis graminum*) infestation in sorghum plants (Peterson 1978; Peters et al., 2009). In addition, several studies have shown sorghum resistance to greenbug infestation in the bloomless phenotypes (Starks & Weibel 1981; Weibel & Starks 1986; Harris-Shultz et al., 2020). However, the presence of EW can also negatively or positively influence the

oviposition and attachment of insects on plants (Gorb et al., 2005; Wójcicka 2016; Eigenbrode & Espelie 1995), thereby affecting the performance of insects on their hosts.

Wójcicka. (2016) found that the feeding behavior of aphids can be a determining factor in the survival and mortality of grain aphids in wax and bloomless triticale plants. The Electrical Penetration Graph (EPG) technique constitutes an effective tool for monitoring SCA feeding behavior in bloomless and wild-type sorghum plants. In this study, we determine if the presence or absence of EW in sorghum plants have any antixenotic or antibiotic effect on SCA populations. Additionally, using the EPG technique we have monitored the feeding behavior of SCA on the wild-type and bloomless plants.

Materials and methods

Plant material

Two sorghum (*Sorghum bicolor*) lines were used in this experiment, both resulting from a cross of *bloomless* Redlan (B-Redlann *bm.*) X *brown midrib* Redlan (B-Redlan *bmr-6*) background. One line is the N104 (Reg. no. GP-253; PI 535789) corresponding to wild-type bloom, which denotes the presence of visual EW and green midribs, and the N106 (Reg. no. GP-255; PI 535791) corresponding to the bloomless with green midribs lacking the presence of epicuticular waxes in aerial parts of the plants (Gorz et al., 1990). Plants were grown until they reached two-weeks-old (3-4 leaf stage) in the University of Nebraska-Lincoln (UNL) greenhouse with a 16-h-light/8-h-dark photoperiod, 25 °C, and 50–60% relative humidity. Seeds were sown in soil mixed with vermiculite and perlite (PRO-MIX BXBIOFUNGICIDE + MYCORRHIZAE, Premier Tech Horticulture Ltd.,

Canada) in Cone-Tainers (Ray Leach SC10; Stuewe & Sons, Inc., Tangent, OR) for the no-choice assay, EPG analysis and wax composition analysis, and in square quart nursery pots (4.5” square x 4.9” deep) for the choice assay. Experiments with wild-type and bloomless were initiated at the same time when plants reached the needed age.

Insect colony

The BCK60 sorghum plants for aphid rearing were grown in the greenhouse until it reached 7-leaf stage. The SCA colony was maintained as previously described (Grover et al., 2020) and was kept on the susceptible BCK60 sorghum genotype in a growth chamber with 16-h-light/8-h-dark photoperiod, $140 \mu\text{E m}^{-2} \text{s}^{-1}$ light quality, 23 °C, and 50–60% relative humidity. Old, deteriorated plants were substituted with new plants in growth chamber whenever necessary. For all the experiments, adult aphids were used and moved to experimental plants with a fine-bristled paintbrush.

No-Choice bioassay

SCA no-choice assay was conducted in the agronomy greenhouse complex at the University of Nebraska-Lincoln, for both wild-type and bloomless sorghum plants. A Completely Randomized Design (CRD) was used to determine the aphid proliferation in both lines. To determine whether SCA proliferation differs between the wild-type and bloomless plants, fifteen (15) plants of each treatment were randomly selected and infested with five (5) adult apterous aphids. Adults were placed in the adaxial side of the second most developed leaf in both lines. After the infestation, plants were caged with tubular clear plastic and ventilated with organdy fabric on the top and sides. The total number of SCA adults and nymphs was counted after 10 days of infestation on each line.

Choice bioassay

SCA choice assay was conducted in the agronomy greenhouse complex at the University of Nebraska-Lincoln, for both wild-type and bloomless sorghum Redland background treatments. For experimental purposes each of the lines were sown in one of the extremes of each square pot. A Completely Randomized Design (CRD) was used to determine the aphid proliferation in both lines. To elucidate whether SCA prefer to settle in the wild-type or the bloomless plants, a total of nineteen (19) pots with two plants in each corner of each treatment were randomly selected for the experiment. Subsequently, 20 adult apterous aphids were put in a small petri dish and then placed in a paper sheet of 40 cm². Aphids were released equidistant to freely move at the same time in all the replications. Square pots were also randomly placed in distinct orientation to avoid air influence bias in the aphid movement. The total SCA adults and nymphs were counted after 1 h, 6 h and 24 h after infestation on the two lines in each of the square pots.

EPG recording

Two-week-old plants were used for the feeding behavior analyses. The experimental procedures and aphid wiring were conducted as described previously (Tetreault et al., 2019). Prior, aphids were starved for 1 h in a plastic petri dish for the EPG recording. Using a stereoscope, a brass nail with a gold wired attached (insect electrode) was glued to dorsum of aphids using a silver conductive glue. After that, a plant electrode (stiff copper wire) was introduced into the soil surrounding the potted plant. For measurements, a Giga-8 EPG model (EPG Systems, Wageningen, The Netherlands) with a 10⁹ Ω resistance amplifier was connected to each of the electrodes and an adjustable

plant voltage were used for measuring feeding behavior of SCA on wild-type and bloomless plants. EPG was conducted at laboratory conditions at 22–24 °C and 40–45% RH under continuous light conditions. Four plants of each line were placed at a time randomly in a Faraday's cage for the recordings. All EPG recordings were initiated between 8 am - 10 am local time (U.S. Central Standard Time). Overall, 14 replications were used for each line (wild-type and bloomless). EPG acquisition software (*Stylet+*, EPG Systems, Wageningen, The Netherlands) was used to record waveforms of SCA feeding. Four categorized EPG waveform phases/patterns were considered in this study: pathway phase, xylem phase, phloem phase and the non-probing phase.

Wax composition analysis

For the wax composition analysis, two-week-old uninfested sorghum plants (wild-type and bloomless) were used for the sample collection. The sample was extracted by carefully placing the second most developed leaf of the plant in a hole puncher of approximately 3 cm² in area, without manipulating or contaminating the collection area. Once the leaf is in the hole puncher near to the tip of the leaf, we punched out one leaf disc. A total of three leaf punches from one plant were considered as one replication in each of the lines and 6 replications were collected for this experiment for each line. The leaf discs were placed directly into a vial of polypropylene cap and polyethylene liner (20 mL 28 x 61 mm (with Cap)) (Busta et al., 2021). The protocol for the analysis of the epicuticular waxes was based as previously described in (Busta et al., 2021) using a GC-MS (gas chromatography and mass spectrometry) system (Agilent 7890A GC).

Statistical analyses

For the no-choice assay and wax composition data analysis, comparisons were performed using a t-test with normal LSD. $\alpha = 0.05$. For the choice assay experiments, the data was transformed by proportions, proportions were calculated by dividing the number of aphids settled in a specific line with the total number of aphids that reached either one of the two tested lines. Data was further analyzed using a Likelihood ratio chi-square (L-R χ^2 test) of independence. EPG data was analyzed using non-parametric Kruskal–Wallis test in four different feeding phases/patterns for each line. Considering the non-normality distribution of the data, the PROC NPAR1WAY procedure was used. Multiple comparisons of different treatments between the means were performed using SAS. Values presented are least square means and standard error.

Results

SCA survival and reproduction did not change between wild-type and bloomless plants

The no-choice assay experiment shows that differences in the SCA aphid survival and reproduction were not significantly different between the wild-type and bloomless plants after 10 days of infestation (Fig. 3.1). SCA aphid population did not differ drastically in both wild-type (average aphid mean population = 482.8) and bloomless (average aphid mean population = 438.6) treatments.

SCA prefer to settle on bloomless plants compared to wild-type plants

The SCA choice-assay shows that the proportion of aphids that reached the bloomless plants was higher compared to the wild-type plants. Significantly higher number of aphids were settled on bloomless plants at 6 h and 24 h after the initial release of aphids, compared to wild-type plants (Fig. 3.2). However, there was no significant difference in the number of aphids that had settled on wild-type vs bloomless after 1 h of aphid release.

SCA spent more time in xylem phase of bloomless plants

Our EPG results revealed that aphids spent longer time in the xylem phase in bloomless plants compared to wild-type plants (Fig. 3.3). No significant differences were found in the pathway phase, phloem phase and non-probing phase between the wild-type and bloomless plants (Fig 3.3).

Bloomless plants have more abundance of 16-monoacylglycerols and 32C-alcohols

Relative abundance ($\mu\text{g}/\text{cm}^2$) of each of the components present in the leaf sample (3 cm^2) between wild-type and bloomless plants is shown in Fig 4. A total of 14 components and one unknown component were detected from the samples collected in wild-type and bloomless plants (Fig. 3.4). The highest abundances were found in 16-monoacylglycerols and 32-C-alcohols in bloomless plants compared to the wild-type plants. No significant differences were observed in the abundance of compounds between wild-type and bloomless plants in the remaining compounds identified in the analysis.

Discussion

This study provides insights into the performance of SCA in sorghum wild-type versus bloomless mutant plants. SCA numbers were not significantly different either in wild-type or bloomless plants in a no-choice assay. However, the aphids preferred to settle on bloomless plants in the choice assays. In addition, SCA spent more time in xylem phase in bloomless plants compared to the wild-type and the wax abundance analysis showed higher amounts of 16-monoacylglycerols and 32-C-alcohols in the bloomless genotype. Our results suggest that lack of waxes in sorghum may not be affecting the SCA proliferation and survival, however, it could be affecting the aphid performance and feeding behavior in sorghum.

Harris-Shultz et al. (2020) have shown that SCA numbers among wax mutants (bloomless) and wild-type plants did not differ considerably, suggesting that the lack of wax components on the surface did not prevent the aphid from reproducing and proliferating on sorghum plants. Similarly, our results show that the presence or absence of waxes does not directly affect the survival of aphids on sorghum. Furthermore, there was no evidence of antibiotic activity related to the lack of EW in sorghum against SCA. However, our study and Harris-Shultz et al. (2020) work contrast with a previous sorghum-greenbug (*Schizaphis graminum*) interactions study, where fewer greenbugs were found on bloomless plants, compared to the wild-type plants (Weibel & Starks, 1986). Interestingly, our choice assay results indicate that SCA preferred to settle on bloomless plants compared to the wild-type plants. Wójcicka (2016) also found that the surface waxes caused feeding deterrence and were toxic to aphids in triticale.

Previously, it was shown that the crystal structures present in the EW disturb the natural movement of insects on plants affecting the natural movement of insects on plants by decreasing the contact area between insect pads and leaves (Gorb. et al., 2005).

Additionally, it was shown that the EW contribute to decreased insect attachment on host plants (Gorb et al., 2005). Our results also suggest that the SCA preferred to settle on bloomless plants, compared to the wild-type plants. One possible explanation is that the aphids could settle and attach better in bloomless plants compared to wild-type plants. Alternatively, the constituents in the EW may deter the aphids in settling on sorghum plants. Waxes can configure an unstable surface for the locomotion of insects (Borodich et al., 2010; Rutledge & Eigenbrode 2003, Yeats & Rose 2013). After 1 h, there was no difference in aphid settlement on wild-type plants compared to bloomless plants.

However, after 6 h and 24 h of initial release of aphids, SCA preferred to settle on bloomless plants, further supporting our hypothesis that constituents present in EW could be influencing the natural aphid attachment and movement on sorghum. In addition, Friedemann et al. (2015) and Gorb & Gorb (2017) showed that crystal structures present in the epicuticular waxes of legumes decreased the attachment force of the pea aphid (*Acyrtosiphon pisum*). Taken together, our results suggest that the wax compounds present in the wild-type plants could contribute to antixenotic responses in sorghum, thereby influencing the SCA behavior.

Our wax analysis displayed a higher amount of long chain alcohols and monoacylglycerols. The fatty alcohols are known to be feeding stimulants for silkworm

(*Bombyx mori*) larvae and chrysomelid beetles, although the feeding behavior of the SCA is different from these insects. The presence of a greater amount of alcohol compounds could be related to the preference by SCA in settling on bloomless plants and additionally to feed more in xylem tissues of bloomless plants compared to the wild-type plants. However, Eigenbrode & Espelie (1995) have shown that the bloomless plants increase the plant's susceptibility to water stress. Given that the aphids prefer to feed more on the xylem tissues of bloomless plants, it is possible that the SCA may encounter this water loss stress more easily in bloomless plants compared to the wild-type plants, however, future experiments need to be performed in terms of leaf water content between wild-type and bloomless plants.

In summary, this study describes impacts of sorghum waxes to SCA performance in sorghum plants. Our results suggests that waxes could play an important role in the antixenotic responses in sorghum against SCA herbivory. Our study provides valuable information that need to be further analyzed and explored. Therefore, this study is important since it denotes basic mechanisms of response in plants to SCA infestation between the wild-type and bloomless plants.

Acknowledgments

I am grateful to Dr. Lucas Busta for his help in analyzing the leaf wax samples and providing advise regarding the wax experiments conducted in this project.

References

- Ayyangar, G. N., Rao, V. P., Nambiar, A. K., & Ponnaiah, B. W. X.** (1937). The occurrence and inheritance of waxy bloom on sorghum. In Proceedings of the Indian Academy of Sciences-Section B (Vol. 5, No. 1, pp. 4-15).
- Borodich, F. M., Gorb, E. V., & Gorb, S. N.** (2010). Fracture behaviour of plant epicuticular wax crystals and its role in preventing insect attachment: a theoretical approach. *Applied Physics A*, 100(1), 63-71.
- Busta, L., Schmitz, E., Kosma, D. K., Schnable, J. C., & Cahoon, E. B.** (2021). A co-opted steroid synthesis gene, maintained in sorghum but not maize, is associated with a divergence in leaf wax chemistry. *Proceedings of the National Academy of Sciences*, 118(12).
- EDDMapS.** (2022). Early Detection & Distribution Mapping System. The University of Georgia - Center for Invasive Species and Ecosystem Health. Available online at <http://www.eddmaps.org/>; last accessed March 13, 2022.
- Eigenbrode, S. D., & Espelie, K. E.** (1995). Effects of plant epicuticular lipids on insect herbivores. *Annual review of entomology*, 40(1), 171-194.
- Espitia-Hernández, P., Chavez Gonzalez, M. L., Ascacio-Valdés, J. A., Dávila-Medina, D., Flores-Naveda, A., Silva, T., Chacón Ruelas, X., & Sepúlveda, L.** (2020). Sorghum (*Sorghum bicolor* L.) as a potential source of bioactive substances and their biological properties. *Critical Reviews in Food Science and Nutrition*, 1-12.
- Friedemann, K., Kunert, G., Gorb, E., Gorb, S. N., & Beutel, R. G.** (2015). Attachment forces of pea aphids (*Acyrtosiphon pisum*) on different legume species. *Ecological Entomology*, 40(6), 732-740.
- Gorb, E. V., & Gorb, S. N.** (2017). Anti-adhesive effects of plant wax coverage on insect attachment. *Journal of Experimental Botany*, 68(19), 5323-5337.
- Gorb, E., Haas, K., Henrich, A., Enders, S., Barbakadze, N., & Gorb, S.** (2005). Composite structure of the crystalline epicuticular wax layer of the slippery zone in the pitchers of the carnivorous plant *Nepenthes alata* and its effect on insect attachment. *Journal of Experimental Biology*, 208(24), 4651-4662.
- Gorz, H. J., Haskins, F. A., & Johnson, B. E.** (1990). Registration of 15 germplasm lines of grain sorghum and sweet sorghum. *Agronomy-Faculty Publications*, 231.
- Harborne, J. B.** (1991). The chemical basis of plant defense. *Plant defenses against mammalian herbivory*, 45.
- Harris-Shultz, K., Punnuri, S., Knoll, J. E., Ni, X., & Wang, H.** (2020). The sorghum epicuticular wax locus Bloomless2 reduces plant damage in P898012 caused by the sugarcane aphid. *Agrosystems, Geosciences & Environment*, 3(1), e20008.

- Louis, J., Lorenc-Kukula, K., Singh, V., Reese, J., Jander, G., & Shah, J.** (2010). Antibiosis against the green peach aphid requires the *Arabidopsis thaliana* MYZUS PERSICAE-INDUCED LIPASE1 gene. *The Plant Journal*, 64(5), 800-811.
- McGinnis, M. J., & Painter, J. E.** (2020). Sorghum: History, use, and health benefits. *Nutrition Today*, 55(1), 38-44.
- Peters, P. J., Jenks, M. A., Rich, P. J., Axtell, J. D., & Ejeta, G.** (2009). Mutagenesis, selection, and allelic analysis of epicuticular wax mutants in sorghum. *Crop science*, 49(4), 1250-1258.
- Peterson, G. C.** (1978). Inheritance of Some Bloomless and Sparse-bloom Mutants in Sorghum (Doctoral dissertation, Oklahoma State University).
- Punnuri, S., & Huang, Y.** (2017). Identification and confirmation of greenbug resistance loci in an advanced mapping population of sorghum. *The Journal of Agricultural Science*, 155(10), 1610-1622.
- Punnuri, S., Harris-Shultz, K., Knoll, J., Ni, X., & Wang, H.** (2017). The genes *bm2* and *blmc* that affect epicuticular wax deposition in Sorghum are allelic. *Crop Science*, 57(3), 1552-1556.
- Rutledge, C. E., & Eigenbrode, S. D.** (2003). Epicuticular wax on pea plants decreases instantaneous search rate of *Hippodamia convergens* larvae and reduces attachment to leaf surfaces. *The Canadian Entomologist*, 135(1), 93-101.
- Singh, B. U., Padmaja, P. G., & Seetharama, N.** (2004). Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. *Crop Protection*, 23(9), 739-755.
- Stamenković, O. S., Siliveru, K., Veljković, V. B., Banković-Ilić, I. B., Tasić, M. B., Ciampitti, I. A., & Prasad, P. V.** (2020). Production of biofuels from sorghum. *Renewable and Sustainable Energy Reviews*, 124, 109769.
- Starks, K. J., & Weibel, D. E.** (1981). Resistance in bloomless and sparse-bloom sorghum to greenbugs. *Environmental entomology*, 10(6), 963-965.
- Tetreault, H. M., Grover, S., Scully, E. D., Gries, T., Palmer, N. A., Sarath, G., Louis, J., & Sattler, S. E.** (2019). Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphid (*Melanaphis sacchari*). *Frontiers in plant science*, 10, 145.
- Uttam, G. A., Praveen, M., Rao, Y. V., Tonapi, V. A., & Madhusudhana, R.** (2017). Molecular mapping and candidate gene analysis of a new epicuticular wax locus in

sorghum (*Sorghum bicolor* L. Moench). Theoretical and Applied Genetics, 130(10), 2109-2125.

Venkateswaran, K., Elangovan, M., & Sivaraj, N. (2019). Origin, domestication, and diffusion of *Sorghum bicolor*. In Breeding Sorghum for diverse end uses, Woodhead Publishing, (pp. 15-31).

Villanueva, R. T., & Sekula, D. (2014). A new pest of sorghum: The sugarcane aphid. In 20th Annual Rio Grande Valley Cotton & Grain Pre-Plant Conference. counties. agrilife.

Walker G.P. (2000). Beginner's guide to electronic monitoring, in Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior, ed. by Walker GP and Backus EA. Entomological Society of America, Lanham, MD, pp. 14–40.

Weibel, D. E., & Starks, K. J. (1986). Greenbug Nonpreference for Bloomless Sorghum 1. Crop Science, 26(6), 1151-1153.

Wójcicka, A. (2016). Effect of epicuticular waxes from triticale on the feeding behaviour and mortality of the grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae). Journal of Plant Protection Research, 56(1).

Yeats, T. H., & Rose, J. K. (2013). The formation and function of plant cuticles. Plant physiology, 163(1), 5-20.

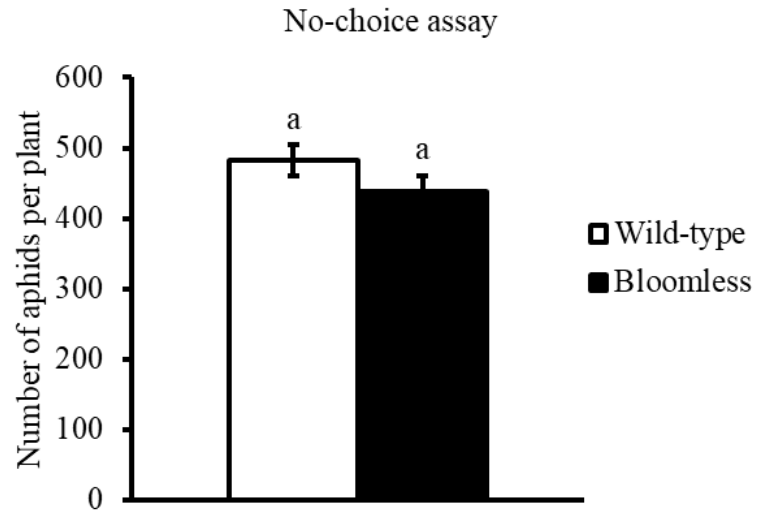


Figure 3.1

Total number of SCA adults and nymphs per plant after 10 days of infestation in wild-type (white bar) and bloomless (black bar) plants. $n = 15$ plants for each treatment. Bars denote the SCA mean pooled number of adults and nymphs per plant in both wild-type and bloomless plants. Same letter above the bars denotes no statistical differences ($P > 0.05$, Tukey's test). Error bars represent \pm SEM.

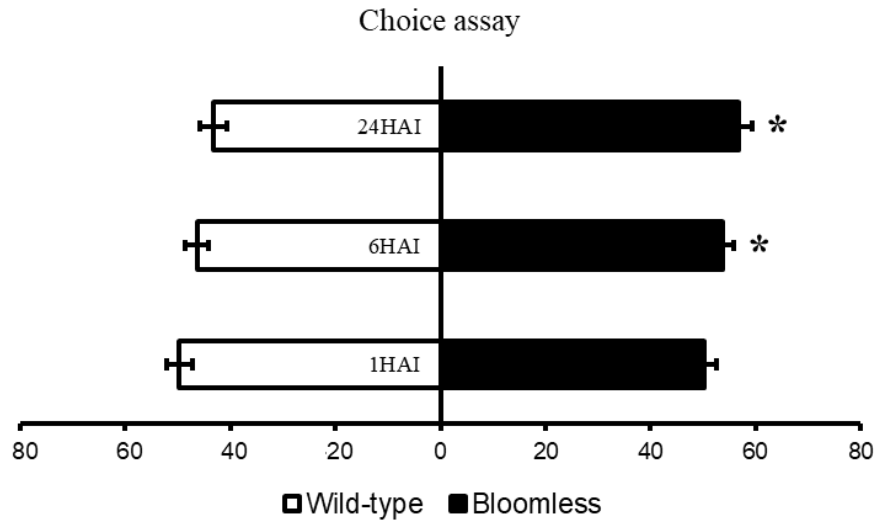


Figure 3.2

Bars represent the proportion of SCA settled on sorghum wild-type and bloomless plants at 1 h, 6 h and 24 h. White bars represent the wild-type plants, black bars represent the bloomless plants. The asterisks indicate significant differences ($P < 0.05$) χ^2 test, $P < 0.05$). Error bars represent \pm SEM.

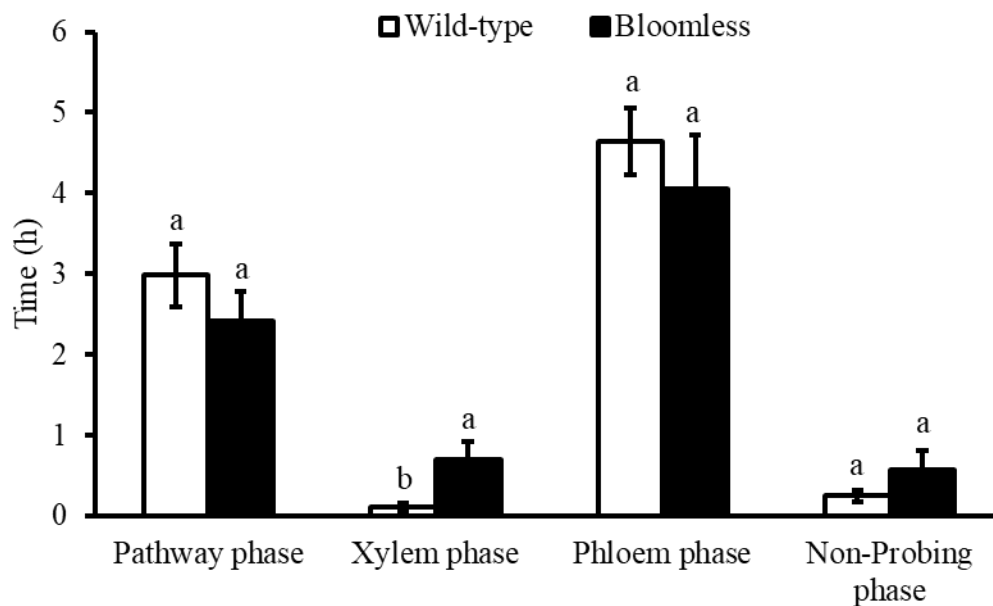


Figure 3.3

Sugarcane aphid (SCA) feeding behavior parameters in wild-type and bloomless plants for the 8 h duration of EPG recording. The total time spent by SCA for different feeding behavior parameters in each of the plant is shown. $n = 14$. Bars denote the mean values obtained for wild-type and bloomless sorghum plants. Bars with different letters denote significant difference (Kruskal-Wallis test; $P < 0.05$). Error bars represent \pm SEM.

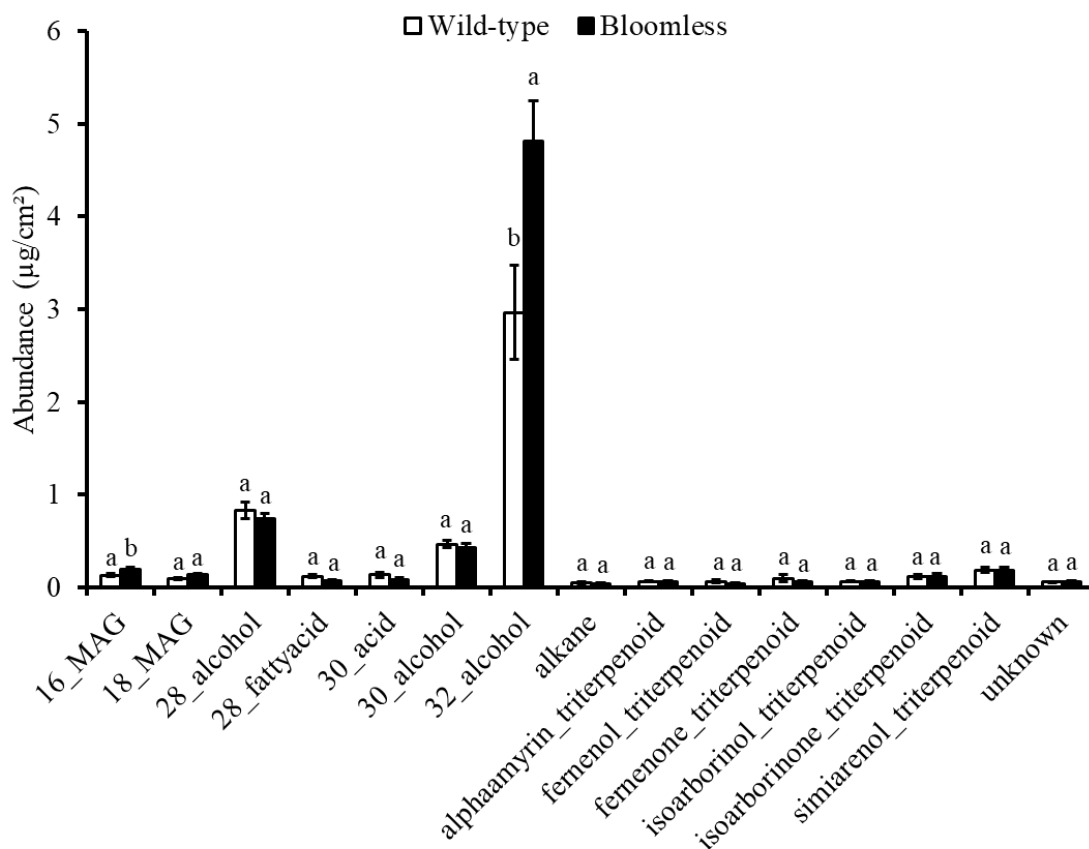


Figure 3.4

Abundance ($\mu\text{g}/\text{cm}^2$) of each of the epicuticular wax components present in the leaf sample (3 cm^2) between sorghum wild-type (white bars) and bloomless (black bars) plants. $n = 6$. Bars with different letter are significantly different from one another based on t-test (normal LSD) test ($\alpha = 0.05$). Error bars represent \pm SEM.