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SHORT COMMUNICATION

# Sorghum cuticular waxes influence host plant selection by aphids

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## Abstract

***Main conclusion*** **Quantification of cuticular waxes coupled with insect bioassays and feeding behavior analysis demonstrate that long-chain $C_{32}$ fatty alcohol impacts host plant selection by aphids.**

**Abstract** Cuticular waxes constitute the first point of contact between plants and their environment, and it also protect plants from external stresses. However, the role of waxes in *Sorghum bicolor* (sorghum) against sugarcane aphid (*Melanaphis sacchari*), a relatively new and devastating pest of sorghum in the U.S., is not fully understood. In this study, we monitored sugarcane aphid behavior on two genotypes of young sorghum plants with different wax chemistry: a wild-type plant (bloom) with lower $C_{32}$ alcohol cuticular wax, and a mutant plant (*bloomless*) with 1.6 times the amount of wax compared to wild-type plants. No-choice aphid bioassays revealed that sugarcane aphid reproduction did not vary between wild-type and the *bloomless* plants. Electrical Penetration Graph (EPG) monitoring indicated that the sugarcane aphids spent comparable amount of time feeding from the sieve elements of the wild-type and *bloomless* plants. However, aphids spent more time feeding on the xylem sap of the *bloomless* plants compared to the wild-type plants. Furthermore, aphid choice assays revealed that the sugarcane aphids preferred to settle on *bloomless* compared to wild-type plants. Overall, our results suggest that cuticular waxes on young sorghum leaves play a critical role in influencing host plant selection by sugarcane aphids.

**Keywords** Cuticular wax · Electrical penetration graph (EPG) · Fatty alcohols · Juvenile leaf · Sorghum · Sugarcane aphid

## Introduction

Sorghum (*Sorghum bicolor*) has many attributes that makes it one of the most utilized and widely cultivated cereals in the world (Venkateswaran et al. 2019). In addition to being grown for animal and human consumption, sorghum is used in the production of biofuels and pharmaceuticals (Stamenković et al. 2020; McGinnis and Painter 2020; Espitia-Hernández et al. 2020). Recently, it has also been shown that sorghum grains can influence the human gut microbiome (Yang et al. 2022). Unfortunately, the sugarcane aphid (*Melanaphis sacchari*) is a major sorghum pest in many parts of the world and sorghum grown regions of the U.S. (Singh et al. 2004; Bowling et al. 2016). To date, sugarcane aphid populations have rapidly increased and have now been reported in 24 states of the U.S. (EDDMapS. 2022). Similar to other aphids, sugarcane aphids are piercing-sucking insects (Singh et al. 2004; Grover et al. 2020, 2022a, b) that ingest plant nutrients by penetrating leaf and stalk tissues using a straw-shaped stylet (Nalam et al. 2019; Zogli et al. 2020). Direct loss of plant nutrients from sugarcane aphid feeding can cause stress, loss of vigor, changes in pigmentation, and plant decline (Bowling et al. 2016; Nibouche et al. 2018). Finally, deposits of aphids' digestive waste, which is the honeydew, on plant surfaces can reduce the photosynthetic capacity and can ultimately lead to plant death and yield loss (Singh et al. 2004; Bowling et al. 2016).

The cuticular waxes constitute the first point of interaction between the aphid and the plant (Eigenbrode and

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Espelie 1995). Moreover, cuticular waxes primarily protect sorghum from desiccation and are associated with sorghum's productivity in dry environments. Before the juvenile-to-adult transition (Hashimoto et al. 2019), sorghum produces leaves with cuticular waxes that are primarily made up of very-long-chain fatty alcohols, together with smaller amounts of fatty acids and alkanes (Busta et al. 2021). In contrast, adults produce leaves with blades that are rich in triterpenoids and adult leaf sheaths bear a thick, visible coating of very-long-chain fatty acids. These thick coatings, or "epicuticular wax blooms", are produced by a variety of crop species and mutants lacking these coatings are referred as *bloomless* plants. Numerous studies have reported that epicuticular wax blooms influence both the performance of sorghum as well as sorghum–insect interactions (Peterson et al. 1982; Starks and Weibel 1981; Weibel and Starks 1986; Peters et al. 2009; Punnuri et al. 2017; Punnuri and Huang 2017). For example, previous studies with sorghum have shown that the absence of epicuticular wax blooms can (i) confer resistance to greenbug (*Schizaphis graminum*) infestation (Peterson et al. 1982; Peters et al. 2009; Starks and Weibel 1981; Weibel and Starks 1986), (ii) influence the oviposition and attachment of insects on plants (Gorb et al. 2005; Wójcicka 2016), and (iii) impact the feeding behavior of aphids, which can be a determining factor in the survival and mortality of grain aphids in wild-type versus *bloomless* triticale plants (Wójcicka 2016).

As described above, sorghum epicuticular wax blooms, which accumulate on the aerial surfaces of adult plants, play a significant role in plant–insect interactions. However, leaves produced by young sorghum plants (three-leaf stage) also have cuticular waxes, but little is known about how these waxes may affect plant–insect interactions. The goal of this study was to assess whether wax chemistry on young sorghum leaves (three-leaf stage) also affects insect survival and behavior.

## Materials and methods

### Plants and growth conditions

Two sorghum lines were used in the study, both resulting from a cross of *bloomless* Redlan (B-Redlan *bm*,) X *brown midrib* Redlan (B-Redlan *bmr*-6) background. N104 (Reg. no. GP-253; PI 535789) corresponds to the wild-type bloom, which denotes the presence of visual epicuticular wax and green midribs, and the other is N106 (Reg. no. GP-255; PI 535791) that corresponds to the *bloomless* with green midribs lacking the presence of visible epicuticular wax on leaf sheaths (Gorz et al. 1990). 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). Plants were grown until they reached two-week-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.

### Insect colony

The BCK60 sorghum plants for aphid rearing were grown in the greenhouse until it reached 7-leaf stage. The sugarcane aphid 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 $\mu$E m$^{-2}$ 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.

### Wax composition analysis

For the wax composition analysis, the sample was extracted by carefully placing the second most developed leaf of the two-week-old plant in a hole puncher of approximately 3 cm$^2$ in area, without manipulating or contaminating the collection area. Once the leaf was 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 for each line and six replications were collected for each line. The leaf discs were placed directly into a vial of polypropylene cap and polyethylene liner (20 mL 28 × 61 mm (with cap)) (Busta et al. 2021). Further, we measured the abundance (in µg/cm$^2$) of wax components present on wild-type and *bloomless* leaf surfaces via gas chromatography–mass spectrometry, as described previously (Busta et al. 2021).

### No-choice bioassays

Sugarcane aphid no-choice assays was conducted for both wild-type and *bloomless* sorghum two-week-old plants in the UNL greenhouse complex. A Completely Randomized Design was used to determine the aphid proliferation on both lines. The wild-type and *bloomless* plants were randomly selected and infested with five adult apterous aphids. Aphids were placed near the bottom of the stem of the plants and plants were caged with tubular clear plastic and ventilated with organdy fabric on the top and sides after infestation. The total numbers of sugarcane aphid adults and nymphs were counted after 10 days of infestation on each line. Aphid no-choice bioassays were conducted twice with similar results.

## Choice bioassays

For choice assay, each sorghum line (wild-type and *bloomless*; two-week-old) was sown in one of the extremes of each square pot (4.5″ square × 4.9″ deep). Twenty adult apterous aphids were introduced at the center of pot on a filter paper of 40 $cm^2$ placed on soil. Aphids were released equidistant from a wild-type and a *bloomless* plant grown in the same pot, so that the sugarcane aphids have the choice of settling on the wild-type or *bloomless* plant. The pots were also randomly placed in distinct orientation to avoid air influence bias in the aphid movement. Aphid choice bioassays were conducted twice with similar results.

## Electrical penetration graph (EPG) recordings

Two-week-old plants were used for the feeding behavior analyses. The experimental procedures and aphid wiring were performed as described previously (Tetreault et al. 2019; Grover et al. 2022a, b). Prior to the beginning of EPG recording, aphids were starved for 1 h in a plastic petri dish. Using a stereoscope, a brass nail with a gold wire (insect electrode) was glued to dorsum of aphids using a silver conductive glue. Subsequently, 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 $10^9$ Ω resistance amplifier was connected to each of the electrode and an adjustable plant voltage were used for measuring feeding behavior of sugarcane aphid on wild-type and *bloomless* plants. EPG was conducted at laboratory conditions at 22–24 °C and 40–45% relative humidity under continuous light conditions. All EPG recordings were initiated between 8 am and 10 am local time (U.S. Central Standard Time). A four-channel GIGA-8 was used for simultaneous recording from four individual aphids on four plants (two channels for the wild-type plant and two for the *bloomless* plant that were placed randomly in a Faraday's cage for the recordings). Overall, 14 replications were performed, and EPG acquisition software (*Stylet*$^+$, EPG Systems, Wageningen, The Netherlands) was used to record waveforms of sugarcane aphid feeding.

## 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 were transformed by proportions, and 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 were further analyzed using a likelihood ratio and Chi-square test of independence. EPG data were 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

First, we determined if there are differences in juvenile leaf wax chemistry of sorghum plants. On juvenile leaves of both wild-type and *bloomless* sorghum plants, we identified a total of 14 compounds including fatty alcohols ($C_{28}$, $C_{30}$, and $C_{32}$), fatty acids ($C_{28}$ and $C_{30}$), an alkane ($C_{31}$), two monoacylglycerides ($C_{16}$ and $C_{18}$), and six triterpenoids (Fig. 1). The fatty alcohols made up nearly all of each mixture (> 78% on both genotypes), but the amount of $C_{32}$ fatty alcohol on the *bloomless* plants was 1.6 times that of wild-type plants (2.96 µg/$cm^2$ on wild-type, 4.81 µg/$cm^2$ on *bloomless*). On both wild-type and *bloomless* surfaces, only a small portion of the wax was unidentifiable (< 1%). Supplemental Figure S1 shows the presence of cuticular waxes in stems of two-week-old wild-type and *bloomless* plants.

Having found that juvenile leaves on wild-type and *bloomless* plants have significantly different surface waxes, we next tested whether this difference caused any changes in sugarcane aphid survival or reproduction. We performed aphid no-choice assays to determine whether sugarcane aphid proliferation differs between the wild-type and *bloomless* plants. No-choice assay revealed that the sugarcane aphid population did not differ between wild-type (average aphid mean population = 482.8) and *bloomless* (average aphid mean population = 438.6) plants (Fig. 2a). This indicates that the differences in wax composition between juvenile leaves on wild-type versus *bloomless* sorghum did not alter sugarcane aphid survival and reproduction.

To study which aphid feeding stages are affected by cuticular waxes, the EPG technique was utilized to compare sugarcane aphid behavior between two-week-old wild-type and *bloomless* plants. Four categorized EPG waveform phases/patterns were considered in this study: pathway phase (inter- and/or intracellular aphid stylet insertion or feeding), xylem phase (aphid feeding on xylem sap and is related to water uptake), sieve element phase (aphid feeding on phloem sap/ingestion of nutrients), and the non-probing phase (fewer or relatively no aphid stylet movement or activity on the plant tissues). Over an 8-h period of EPG recording, we found no significant differences in the pathway, sieve element and non-probing phases between the wild-type and *bloomless* plants (Fig. 2b). However, EPG results revealed that the sugarcane aphids spent significantly longer time in

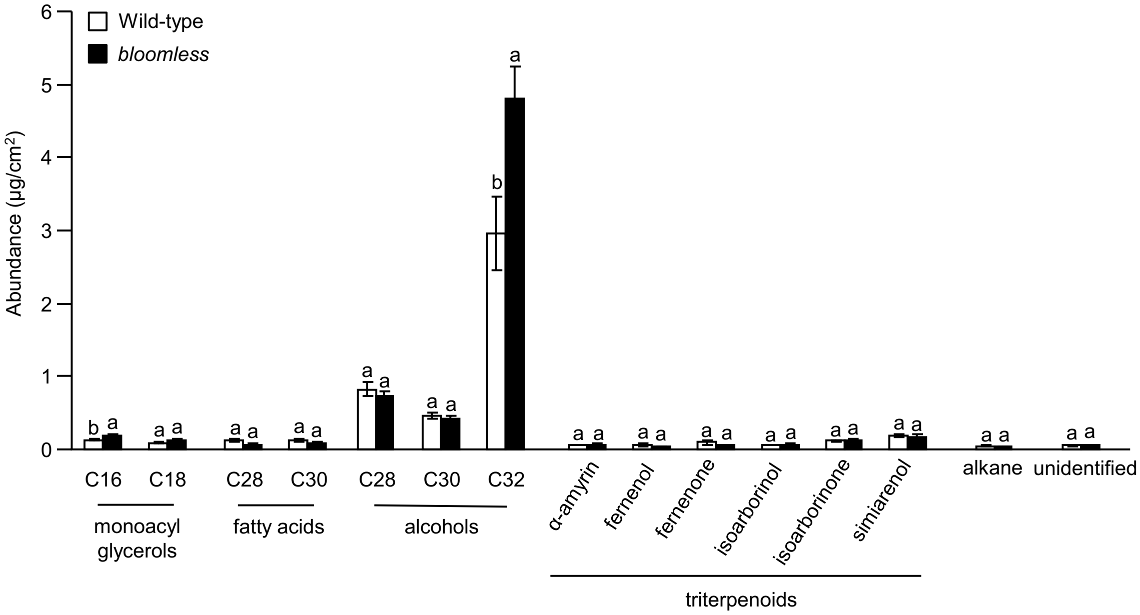

**Fig. 1** Wax chemistry on leaves of juvenile wild-type and *bloomless* sorghum plants. Abundance (μg/cm$^2$) of each of the cuticular wax components present on the leaf sample (3 cm$^2$) between sorghum wild-type (white bars) and *bloomless* (black bars) plants ($n = 6$). Bars with different letters are significantly different from one another based on pairwise $t$ tests (normal LSD) test ($\alpha = 0.05$). Error bars represent mean ± SEM

the xylem phase of *bloomless* plants compared to wild-type plants (Fig. 2b).

To determine whether cuticular waxes contribute to host plant selection by aphids, host choice by the aphid was studied on the wild-type and *bloomless* plants. The total number of adult sugarcane aphids that were settled on each plant were counted after 1, 6, and 24 h of aphid release. There was no significant difference in the number of aphids that had settled on wild-type vs *bloomless* plants 1 h after aphid release (Fig. 3). However, sugarcane aphids preferred to settle on *bloomless* plants compared to the wild-type plants after 6 and 24 h of initial release of aphids (Fig. 3). This indicates that sugarcane aphids preferred to settle on *bloomless* plants compared to wild-type plants.

## Discussion

Collectively, our study provides insights into the performance of sugarcane aphid on young (three-leaf stage) sorghum wild-type vs. *bloomless* plants. Sugarcane aphid 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 compared to the wild-type plants in the choice assays. In addition, sugarcane aphid spent more time in xylem phase in *bloomless* plants compared to the wild-type plants. Further, the wax component analysis showed higher amounts of 16-monoacylglycerols and 32-C-alcohols in the *bloomless* genotype. Together, our results suggest that cuticular waxes in young sorghum plants (three-leaf stage) may not be affecting the sugarcane aphid proliferation and survival; however, it may affect the aphid performance and feeding behavior.

Harris-Shultz et al. (2020) have shown that sugarcane aphid numbers among wax mutants (*bloomless*) and wild-type plants did not differ considerably, which suggested that the lack of wax components on the surface did not prevent the aphid from reproducing and proliferating on sorghum plants. Those results align with our results confirming 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, which limits insect population, related to the lack of cuticular waxes in sorghum against sugarcane aphid. 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 and Starks 1986). Interestingly, our choice assay results indicate that sugarcane aphid preferred to settle on *bloomless* plants compared to the wild-type plants. Wójcicka (2016) also found that the surface waxes, which deterred feeding in triticale, were toxic to aphids.

The crystal structures of epicuticular waxes may disturb the normal movements of insects on plant epidermis by decreasing the contact area between insect pads and leaf

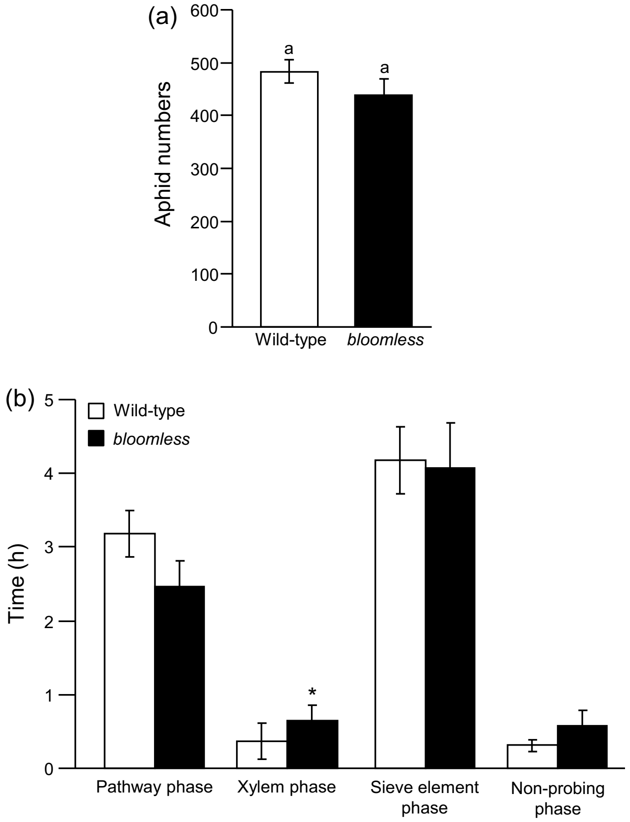

**Fig. 2** Sugarcane aphid performance and feeding on leaves of juvenile wild-type and *bloomless* sorghum plants. **a** Total number of sugarcane aphid adults and nymphs per plant after 10 days of infestation in wild-type (white bar) and *bloomless* (black bar) plants ($n = 15$). Error bars represent mean ± SEM. Same letter above the bars denotes no statistical differences ($P > 0.05$, Tukey's test). Aphid no-choice experiments were conducted twice with similar results. **b** Electrical Penetration Graph (EPG) monitoring of mean time spent by sugarcane aphid for various feeding behavior activities over an 8 h feeding on wild-type and *bloomless* plants ($n = 14$). An asterisk (*) represents significant difference ($P < 0.05$; Kruskal–Wallis test) in the time spent by sugarcane aphid for the indicated activity on the wild-type and *bloomless* plants

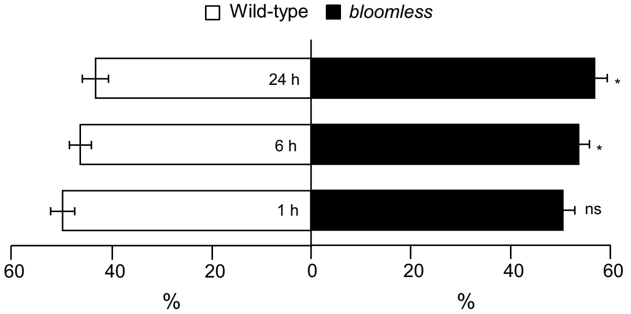

**Fig. 3** Choice assay comparison of sugarcane aphid feeding on leaves of juvenile wild-type and *bloomless* sorghum plants. Twenty adult sugarcane aphids were released at the center of a pot containing one plant of each wild-type and *bloomless* sorghum plant. Proportion of adult sugarcane aphids that had settled on each plant combination were monitored after 1, 6, and 24 h post-aphid release ($n = 19$). An asterisk (*) indicate values that are significantly different from each other ($P < 0.05$; $\chi^2$ test), while "ns" indicates no significant differences between sorghum plants on sugarcane aphid settling. Aphid choice experiments were conducted twice with similar results

surface (Gorb et al. 2005). Additionally, epicuticular waxes were shown to contribute to decreased insect attachment on host plants (Gorb et al. 2005). Our results also suggest that the sugarcane aphid preferred to settle on *bloomless* plants compared to the wild-type plants. One possible explanation for this result is that the aphids have a better mobility on *bloomless* plants compared to wild-type plants. Alternatively, the constituents of the cuticular waxes may also deter the aphids in settling on sorghum plants. Waxes can constitute an unstable surface for insect locomotion (Borodich et al. 2010; Rutledge and Eigenbrode 2003; Yeats and Rose 2013). Our results show that there was no difference in aphid settlement on wild-type plants compared to *bloomless* plants after 1 h of aphid release. However, after 6 and 24 h of initial aphid release, sugarcane aphids preferred to settle on *bloomless* plants, further supporting our hypothesis that constituents present in cuticular waxes could be influencing the natural aphid attachment and movement on sorghum. In addition, Friedemann et al. (2015) and Gorb and Gorb (2017) demonstrated that crystal structures present in the cuticular waxes of legumes decreased the attachment force of the pea aphid (*Acyrthosiphon pisum*). Taken together, our results suggest that the wax compounds present on the surfaces of wild-type plants could contribute to antixenotic responses, which deters aphid settling in sorghum, thereby influencing the sugarcane aphid behavior.

Our wax analysis displayed a higher amount of long-chain alcohols on *bloomless* plants compared with the wild-type plants. The fatty alcohols are known to be feeding stimulants for silkworm (*Bombyx mori)* larvae and chrysomelid beetles (Mori 1982; Adati and Matsuda 1993), although these insects have a different feeding behavior than sugarcane aphids. The presence of a greater amount of alcohol compounds in *bloomless* plants could be related to the observed settling preference and increased feeding time by sugarcane aphids in xylem tissues of *bloomless* plants compared to the wild-type plants. It was also suggested that the alterations in epicuticular wax contents can influence the plant's susceptibility to water stress (Cole and Riggall 1993). Given that the sugarcane aphids prefer to feed more on the xylem tissues of *bloomless* plants, it is plausible that the sugarcane aphids may encounter water stress more frequently in *bloomless* plants compared to the wild-type plants. It has also been suggested that aphids consume more water from the xylem tissues to dilute the sugar content in the gut, which is a strategy used by aphids to maintain their water balance (Spiller et al. 1990; Pompon et al. 2010). Future experiments need to be performed to quantify the leaf water and sugar contents between the wild-type and *bloomless* plants.

In summary, this study describes impacts of sorghum waxes to sugarcane aphid performance in sorghum plants. Our results suggest that waxes could play an important role in the antixenotic responses in sorghum against sugarcane aphid herbivory. Additionally, our study helps to tease apart how cuticular waxes influence host plant selection by aphids on sorghum plants and provides valuable insights on juvenile leaf wax chemistry of sorghum plants.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00425-022-04046-3.

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**Author contributions** JBC and JL conceived the research. JBC, SG, LB, and JL designed experiments. JBC, SG, and LB conducted the experiments, and collected and analyzed the data. SES contributed reagents and provided guidance on experiments. JBC and JL wrote the first draft of the manuscript. All authors reviewed and edited the manuscript.

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**Data availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

## References

Adati T, Matsuda K (1993) Feeding stimulants for various leaf beetles (Coleoptera: Chrysomelidae) in the leaf surface wax of their host plants. Appl Entomol Zool 28:319–324. https://doi.org/10.1303/aez.28.319

Borodich FM, Gorb EV, Gorb SN (2010) Fracture behaviour of plant epicuticular wax crystals and its role in preventing insect attachment: a theoretical approach. Appl Phys A 100:63–71. https://doi.org/10.1007/s00339-010-5794-x

Bowling RD, Brewer MJ, Kerns DL, Gordy J, Seiter N, Elliott NE, Buntin GD, Way MO, Royer TA, Biles S, Maxson E (2016) Sugarcane aphid (Hemiptera: Aphididae): A new pest on sorghum in North America. J Integr Pest Manag 7:1–13. https://doi.org/10.1093/jipm/pmw011

Busta L, Schmitz E, Kosma DK, Schnable JC, Cahoon EB (2021) A co-opted steroid synthesis gene, maintained in sorghum but not maize, is associated with a divergence in leaf wax chemistry. Proc Natl Acad Sci (USA) 118:e2022982118. https://doi.org/10.1073/pnas.2022982118

Cole RA, Riggall W (1992) Pleiotropic effects of genes in glossy *Brassica oleracea* resistant to *Brevicoryne brassicae*. In: Menken SBJ, Visser JH, Harrewijn P (eds) Proceedings of the 8th International Symposium on Insect-Plant Relationships. Springer Netherlands, Dordrecht, pp 313–315

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/

Eigenbrode SD, Espelie KE (1995) Effects of plant epicuticular lipids on insect herbivores. Ann Rev Entomol 40:171–194. https://doi.org/10.1146/annurev.en.40.010195.001131

Espitia-Hernández P, Chávez González ML, Ascacio-Valdés JA, Dávila-Medina D, Flores-Naveda A, Silva T, Chacón Ruelas X, Sepúlveda L (2022) Sorghum (*Sorghum bicolor* L.) as a potential source of bioactive substances and their biological properties. Crit Rev Food Sci Nutr 62:2269–2280. https://doi.org/10.1080/10408398.2020.1852389

Friedemann K, Kunert G, Gorb E, Gorb SN, Beutel RG (2015) Attachment forces of pea aphids (*Acyrthosiphon pisum*) on different legume species. Ecol Entomol 40:732–740. https://doi.org/10.1111/een.12249

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. J Exp Biol 208:4651–4662. https://doi.org/10.1242/jeb.01939

Gorb EV, Gorb SN (2017) Anti-adhesive effects of plant wax coverage on insect attachment. J Exp Bot 68:5323–5337. https://doi.org/10.1093/jxb/erx271

Gorz HJ, Haskins FA, Johnson BE (1990) Registration of 15 germplasm lines of grain sorghum and sweet sorghum. Crop Sci 30:762–763. https://doi.org/10.2135/cropsci1990.0011183X003000030089x

Grover S, Agpawa E, Sarath G, Sattler SE, Louis J (2020) Interplay of phytohormones facilitate sorghum tolerance to aphids. Plant Mol Biol. https://doi.org/10.1007/s11103-020-01083-y

Grover S, Cardona JB, Zogli P, Alvarez S, Naldrett MJ, Sattler SE, Louis J (2022a) Reprogramming of sorghum proteome in response to sugarcane aphid infestation. Plant Sci 320:111289. https://doi.org/10.1016/j.plantsci.2022.111289

Grover S, Puri H, Xin Z, Sattler SE, Louis J (2022b) Dichotomous role of jasmonic acid in modulating sorghum defense against aphids. Mol Plant Microbe Interact 35:755–767. https://doi.org/10.1094/MPMI-01-22-0005-R

Harris-Shultz K, Punnuri S, Knoll JE, Ni X, Wang H (2020) The sorghum epicuticular wax locus *Bloomless2* reduces plant damage in P898012 caused by the sugarcane aphid. Agrosyst Geosci Environ 3:e20008. https://doi.org/10.1002/agg2.20008

Hashimoto S, Tezuka T, Yokoi S (2019) Morphological changes during juvenile-to-adult phase transition in sorghum. Planta 250:1557–1566. https://doi.org/10.1007/s00425-019-03251-x

McGinnis MJ, Painter JE (2020) Sorghum: History, use, and health benefits. Nutr Today 55:38–44. https://doi.org/10.1097/NT.0000000000000391

Mori M (1982) n-Hexacosanol and n-octacosanol: Feeding stimulants for larvae of the silkworm, *Bombyx mori*. J Insect Physiol 28:969–973. https://doi.org/10.1016/0022-1910(82)90114-7

Nalam V, Louis J, Shah J (2019) Plant defense against aphids, the pest extraordinaire. Plant Sci 279:96–107. https://doi.org/10.1016/j.plantsci.2018.04.027

Nibouche S, Costet L, Holt JR, Jacobson A, Pekarcik A, Sadeyen J, Armstrong JS, Peterson GC, McLaren N, Medina RF (2018) Invasion of sorghum in the Americas by a new sugarcane aphid

(*Melanaphis sacchari*) superclone. PLoS ONE. https://doi.org/10.1371/journal.pone.0196124

Peters PJ, Jenks MA, Rich PJ, Axtell JD, Ejeta G (2009) Mutagenesis, selection, and allelic analysis of epicuticular wax mutants in sorghum. Crop Sci 49:1250–1258. https://doi.org/10.2135/cropsci2008.08.0461

Peterson GC, Suksayretrup K, Weibel DE (1982) Inheritance of some bloomless and sparse-bloom mutants in sorghum. Crop Sci 22:63–67. https://doi.org/10.2135/cropsci1982.0011183X002200010014x

Pompon J, Quiring D, Giordanengo P, Pelletier Y (2010) Role of xylem consumption on osmoregulation in *Macrosiphum euphorbiae* (Thomas). J Insect Physiol 56:610–615. https://doi.org/10.1016/j.jinsphys.2009.12.009

Punnuri S, Harris-Shultz K, Knoll J et al (2017) The Genes *Bm2* and *Blmc* that affect epicuticular wax deposition in sorghum are allelic. Crop Sci 57:1552–1556. https://doi.org/10.2135/cropsci2016.11.0937

Punnuri S, Huang Y (2017) Identification and confirmation of greenbug resistance loci in an advanced mapping population of sorghum. J Agric Sci 155:1610–1622. https://doi.org/10.1017/S0021859617000685

Rutledge CE, Eigenbrode SD (2003) Epicuticular wax on pea plants decreases instantaneous search rate of *Hippodamia convergens* larvae and reduces attachment to leaf surfaces. Can Entomol 135:93–101. https://doi.org/10.4039/n02-044

Singh BU, Padmaja PG, Seetharama N (2004) Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. Crop Prot 23:739–755. https://doi.org/10.1016/j.cropro.2004.01.004

Spiller NJ, Koenders L, Tjallingii WF (1990) Xylem ingestion by aphids–a strategy for maintaining water balance. Entomol Exp Appl 55:101–104. https://doi.org/10.1111/j.1570-7458.1990.tb01352.x

Stamenković OS, Siliveru K, Veljković VB et al (2020) Production of biofuels from sorghum. Renew Sust Energ Rev 124:109769. https://doi.org/10.1016/j.rser.2020.109769

Starks KJ, Weibel DE (1981) Resistance in bloomless and sparse-bloom sorghum to greenbugs. Environ Entomol 10:963–965. https://doi.org/10.1093/ee/10.6.963

Tetreault HM, Grover S, Scully ED, Gries T, Palmer NA, Sarath G, Louis J, Sattler SE (2019) Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphid (*Melanaphis sacchari*). Front Plant Sci 10:145. https://doi.org/10.3389/fpls.2019.00145

Venkateswaran K, Elangovan M, Sivaraj N (2019) Chapter 2 - Origin, domestication and diffusion of *Sorghum bicolor*. In: Aruna C, Visarada KBRS, Bhat BV, Tonapi VA (eds) Breeding sorghum for diverse end uses. Woodhead Publishing, pp 15–31

Weibel DE, Starks KJ (1986) Greenbug nonpreference for bloomless sorghum. Crop Sci. https://doi.org/10.2135/cropsci1986.0011183X002600060014x

Wójcicka A (2016) Effect of epicuticular waxes from triticale on the feeding behaviour and mortality of the grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae). J Plant Prot Res 56:39–44. https://doi.org/10.1515/jppr-2016-0006

Yang Q, Van Haute M, Korth N et al (2022) Genetic analysis of seed traits in *Sorghum bicolor* that affect the human gut microbiome. Nat Commun 13:5641. https://doi.org/10.1038/s41467-022-33419-1

Yeats TH, Rose JKC (2013) The formation and function of plant cuticles. Plant Physiol 163:5–20. https://doi.org/10.1104/pp.113.222737

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. Curr Opin Plant Biol 56:153–161. https://doi.org/10.1016/j.pbi.2020.06.002