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Nymphal Performance and Fecundity of *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) in Different Sorghum Genotypes

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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is a tropical grass native to the African continent that is often used as human and animal feed. Polyphagous insects such as the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), have benefited from the increase of sorghum planted in Brazil, the United States (US), and around the world. This study evaluated the performance of nymphs and adult fecundity of sugarcane aphids in 12 sorghum genotypes to find sources of resistance to the aphid while considering the current lack of efficient control methods that are less aggressive to the environment. Each repetition consisted of a single sorghum seedling infested with 10 nymphs in the first instar, in no-choice tests, totaling 10 replicates per genotype. The pre-reproductive period, nymphal period, daily and total nymph production, nymphal viability, percentage of adult emergence, and wax content were evaluated. After five days of confinement, the plants were evaluated, counting the number of adults present in the seedlings. All genotypes compromised the biological performance of *M. sacchari.* The genotypes 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207 induced 100% of nymphal mortality. No difference was found between genotypes in the wax content on the sorghum leaves. These results are important for the species *M. sacchari* and may provide subsidies for future breeding programs, focusing on sorghum resistance to aphids.

Keywords: Sugar cane aphid, *Sorghum bicolor*, Host-plant resistance, nymphoposition.

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a tropical grass native to the African continent and is very versatile in its uses. These uses range from human and animal feed to raw material producing anhydrous alcohol, alcoholic beverages, glues, paints, brooms, and sugar extraction. It is becoming increasingly popular in the food industry for its gluten-free components [1, 2].

The estimated sorghum-planted area in Brazil for the 2021-2022 years is approximately 841.3 thousand hectares, with expected production of 2.4 million tons [3]. In the United States, sorghum is the fourth largest crop in production, behind only the major commodities, corn (*Zea mays L.*), soybean [*Glycine max* (L.) Merrill] and wheat (*Triticum aestivum* L.). The major sorghum-producing states are in the central and southern Great Plains regions, with 65% of production in Kansas and Texas [4, 5].

The sorghum crop stands out in Brazilian agriculture because it can adapt to adverse environmental situations of abiotic stresses, such as low rainfall rates, and low air and soil humidity. This adaptability contributes significantly to minimizing problems in the fall season [6]. Polyphagous insects such as the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), benefit from the increased area of planted sorghum in Brazil [3]. The crop is used as a green bridge and allows the survival of nymphs and adults of *M. sacchari*,

multiplying the population and increasing the damage caused by the colony for future harvests. *Melanaphis sacchari* is a major pest of sorghum in Africa, Asia, Australia, and the US, and was introduced in Brazil in the 1970s [7]. The sorghum plant can be attacked immediately after seedling emergence, but significant infestations occur mainly during drier periods of the year [8]. The sugarcane aphid feeds on the leaf sap on the abaxial surface causing damage to the plants that includes purple coloration of the seedlings, followed by chlorosis, necrosis, delayed flowering, poor grain filling, and losses in quality and quantity of the grains [7, 9]. In the leaves below the already colonized ones, they are covered by honeydew and allow the fungi colonization of the genus *Capnodium* spp. thereby hindering the breathing and photosynthesis of the plant [7]. Besides the damage caused by the introduction of the mouth apparatus for feeding, *M. sacchari* can transmit three types of viruses, *millet red leaf virus* [10], *sugarcane yellow leaf virus* in sorghum and sugarcane [11], and *sugarcane mosaic virus* (SMV) in sorghum [12].

The main control method employed for the aphid *M. sacchari* in sorghum crops is spraying insecticides of synthetic origin [9]. The indiscriminate use of these insecticides and mixtures of active ingredients can cause irreversible impacts on the environment, harm non-target organisms, alter insect population levels in the agro-ecosystem [13, 14], and select resistant individuals, reducing the efficiency of this controlled practice [15]. The use of insect-resistant cultivars can be an efficient alternative to reducing the use of insecticides in crops, besides being compatible with the other tactics prescribed in Integrated Pest Management (IPM). The plant resistance to certain pest species is related to their physical, chemical, and morphological characteristics, which can change the preference and biology of the insect, contributing to the reduction of its population and keeping it at levels that do not cause economic losses. This contributes to the conservation of the agro-ecosystem and provides greater profitability to the producer [16].

This study aimed to evaluate the biological parameters of the sugarcane aphid confined to 12 sorghum genotypes under laboratory conditions. Because of the rising importance of sorghum cultivation in Brazil, the US, and the world, and the expressive damage potential that *M. sacchari* and associated pathogens present to the sorghum crop, it is important to investigate this subject for future food production and/or increasing producer profitability

MATERIALS AND METHODS

The study was performed in climate-controlled chambers (T = 23 ± 1 ºC, R.H. = 65 ± 10% and photoperiod = 14 h), located in Hays, KS, United States, (38º 51' N, 99º 20' W) at an Agricultural Research Center, Kansas State University (KSU) during 2017.

Breeding sugarcane aphids (Melanaphis saccari)

A colony of *M. sacchari* was maintained in sorghum seedlings (susceptible commercial hybrid, cv. P85Y40 (Dupont-Pioneer, Johnston, IA) in a climate-controlled growth chamber (T = 23 ± 1 ºC, R.H. = 65 ± 10%, and photoperiod = 14 h). Sorghum seeds were planted in metal trays (8.0 by 51.0 by 36.0 cm) containing a mixture of soil, peat moss, and vermiculite (1:1:1) and germinated in a growth chamber set to the same temperature and photoperiod as above. Seedlings were watered daily until they were 4.0 to 8.0-cm tall (three- to the four-leaf stage) before being infested with aphids. New aphid colonies were started weekly by clipping infested leaves and placing them on fresh trays of sorghum seedlings. Care was taken to ensure experimental aphids were collected only from low-density colonies to minimize the possibility of wing development.

Bioassay

Each experimental replicate consisted of a single sorghum seedling germinated in a 16.0-cm plastic cone (Stuewe & Sons, Corvallis, OR) filled with a mixture of soil, peat moss, and vermiculite (1:1:1) under the same physical conditions as plants for the aphid colonies. Three seeds were germinated in each cone and the resulting seedlings thinned to leave a single, vigorous plant. The plants were infested with 10 nymphs of *M. sacchari* when they were with 5.0-cm tall (two–three-leaf stage, 5–6 d post-germination), totaling 10 replicates per treatment. Following aphid infestation, each cone was covered with a custom-made, clear plastic cylinder (30 cm long),

sealed at the top with a plastic plug, and ventilated on the sides using a series of holes covered with a fine mesh. All cones were held in a supporting rack and placed in a growth chamber (T = 23 ± 1 ºC, R.H. = 65 ± 10% e photoperiod =14 h). Plants were watered every 48 h by submerging the rack of cones in a water bath for 30 min.

The assessments of aphid performance have typically involved the observation of solitary aphid nymphs developing in clip cages, and the daily harvesting of nymphs to tally their fecundity [17]. However, because many aphid species are naturally gregarious, solitary development and reproduction may represent hardship and can potentially lead to underestimates of biological performance. Solitary aphids will be at a disadvantage whenever benefits of group-feeding (e.g., sink effects) occur in natural colonies [18]. Due to the gregarious behavior of the sugarcane aphid, the methodology used in this bioassay better approximates natural conditions as the nymphs develop in groups rather than in isolation.

Therefore, we evaluated the pre-reproductive period, nymphal period, daily and total nymph production up to 10 days, nymphal survival, and percentage of adult emergence. After five days of confinement, the plants were evaluated by counting the number of adults present in the seedlings. This evaluation was done daily until the last nymph became an adult, leaving it to reproduce on the plant for 10 days.

Wax analysis

To characterize the adaxial and abaxial epicuticular wax layer of the sorghum leaves, five plants (V3-V4) of each treatment were used, one plant being considered a repetition. All leaves were removed with scissors and the collected samples were submerged separately for 20 s in a beaker (200 ml) with 50 ml of chloroform, which had been previously weighed, and the leaves were gently shaken. The solutions obtained (wax + chloroform) evaporated in an exhaust hood to obtain the solid residue (wax). After complete evaporation, the beakers were reweighed and the wax content was determined as a function of the mass difference [17].

Statistical analysis

The data were subjected to an analysis of variance, with normality and homoscedasticity verified with the Shapiro-Wilk and Levene's test. When significant differences in the effects of the treatments were verified, a Tukey test was used ($P \leq 0.05$) to compare the means, using the statistical program R 3.2.1. version [19].

RESULTS AND DISCUSSION

Among the 12 sorghum genotypes evaluated, aphids confined in five of them (PI 550610, 2840B, BH 3400, W7051, and W844E) were able to complete the nymphal stage and reach the adult stage **(Table 1)**. Nymphs on the other seven genotypes (84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207) did not complete the immature stage. Therefore, these genotypes caused 100% mortality **(Table 1)**.

Table 1. Mean number (± EP) of the pre-reproductive period, nymphal period, nymphs per day, and total nymphs of *Melanaphis sacchari* in 12 sorghum genotypes under controlled conditions. Hays-KS, 2017.

Genotype	Pre-reproductive period (d)[1]	Nymphal period (d)[1]	Nymphs/adult/d[1]	Total of nymphs[1]
PI 550610	1.16 ± 0.28 (n[3] = 6)	7.92 ± 0.58 (n = 10) b	2.71 ± 0.45 (n = 6) a	27.17 ± 4.52 (n = 6) a
2840B	1.33 ± 0.22 (n = 4)	7.37 ± 0.17 (n = 5) b	2.17 ± 0.18 (n = 4) ab	21.75 ± 1.75 (n = 4) ab
BH 3400	2.87 ± 0.44 (n = 9)	8.58 ± 0.47 (n = 9) ab	1.62 ± 0.15 (n = 9) ab	16.22 ± 1.54 (n = 9) b
W7051	3.28 ± 0.71 (n = 7)	11.05 ± 1.42 (n = 8) a	1.18 ± 0.21 (n = 7) b	11.85 ± 2.11 (n = 7) b
W844E	-	14,00[2] a	-	-
84P68	-	-	-	-
CHR 2042	-	-	-	-
DKS 3707	-	-	-	-
HG35W	-	-	-	-
M60GB31	-	-	-	-
SP73B12	-	-	-	-

95207	-	-	-	-
P	0,0604	0,0478	0,0387	0,0387

[1]Means followed by the same letter in each column do not differ by the Tukey test ($P \geq 0,05$). [2]Only one insect was obtained. [3]n= number of evaluated insects.

There was no statistical difference between treatments regarding the pre-reproductive period, with means ranging from 1.16 to 3.28 days **(Table 1)**. The W844E (14.00 days) and W7051 genotypes (11.05 days) were the ones that prolonged the nymphal period, differing statistically from the 2840B (7.37 days) and PI 550610 genotypes (7.92 days) that presented the lowest mean values for the immature phase period of the insect **(Table 1)**.

Regarding the production of nymphs per day, the W7051 genotype (1.18 nymphs) showed the lowest mean number of nymphs, differing from the PI 550610 genotype (2.71 nymphs). Of the aphids confined in the W844E genotype, only one insect reached the adult stage. That aphid did not produce nymphs and it was not possible to collect data for this parameter **(Table 1)**. Regarding the mean production of nymphs in 10 days, the W7051 (11.85 nymphs), and BH 3400 genotype (16.22 nymphs) showed the lowest values differing statistically from the PI 550610 genotype (27.17 nymphs) **(Table 1)**. Still, regarding the mean number of nymphs during the 10 days of nymphoposition, it was observed that the peak production of nymphs for each genotype was between 5 and 7 days **(Figure 1)**. After this period, there was a decrease in offspring production in all genotypes **(Figure 1)** until the last day of the evaluation (10 days).

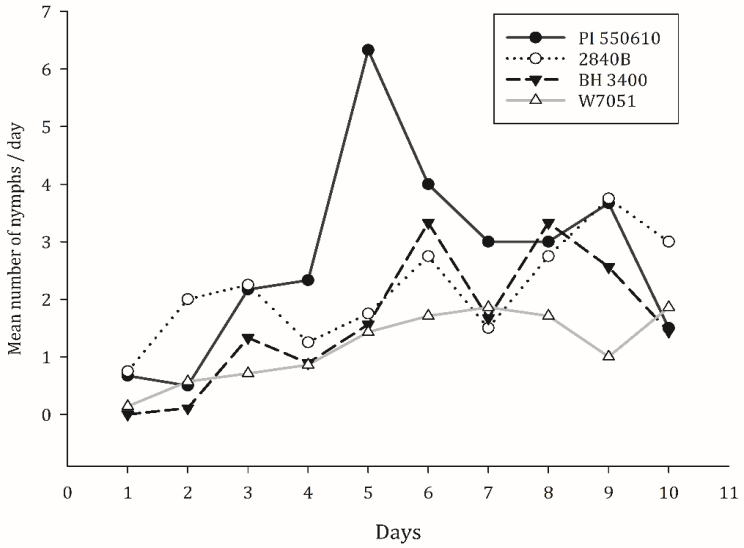


Figure 1. The mean number of *Melanaphis sacchari* nymphs observed in four sorghum genotypes during the reproductive period per each reproductive day under controlled conditions. Hays-KS, 2017.

The nymphal survival ranged from zero to 58%, with highlights for 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, 95207 (0%), and W844E genotypes (1%) **(Figure 2)**. The aphids confined in the W844E genotype showed the lowest mean daily percentage of adult emergence, 14 days to reach 1% of emergence **(Figure 3)**. The W7051 genotype showed adult emergence from the first evaluation (5 days) until the 11th day and reached the maximum mean value of adult emergence at 10 days (8.00 %) **(Figure 3)**. The 2840B and BH 3400 genotype also allowed the emergence of adults since the first day of evaluation, reaching the maximum mean of adult emergence at seven days, 14 and 20%, respectively **(Figure 3)**. The PI 550610 genotype allowed the highest mean number of adults emerging at 8 days (36.25 %) **(Figure 3)**.

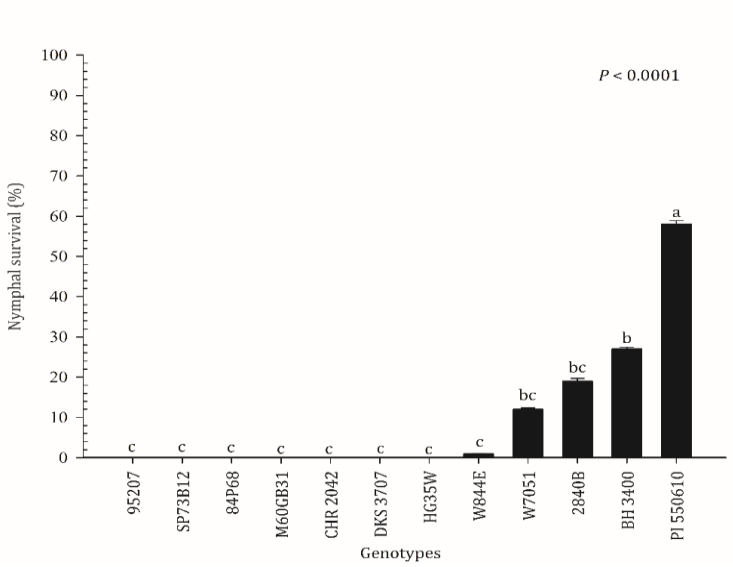
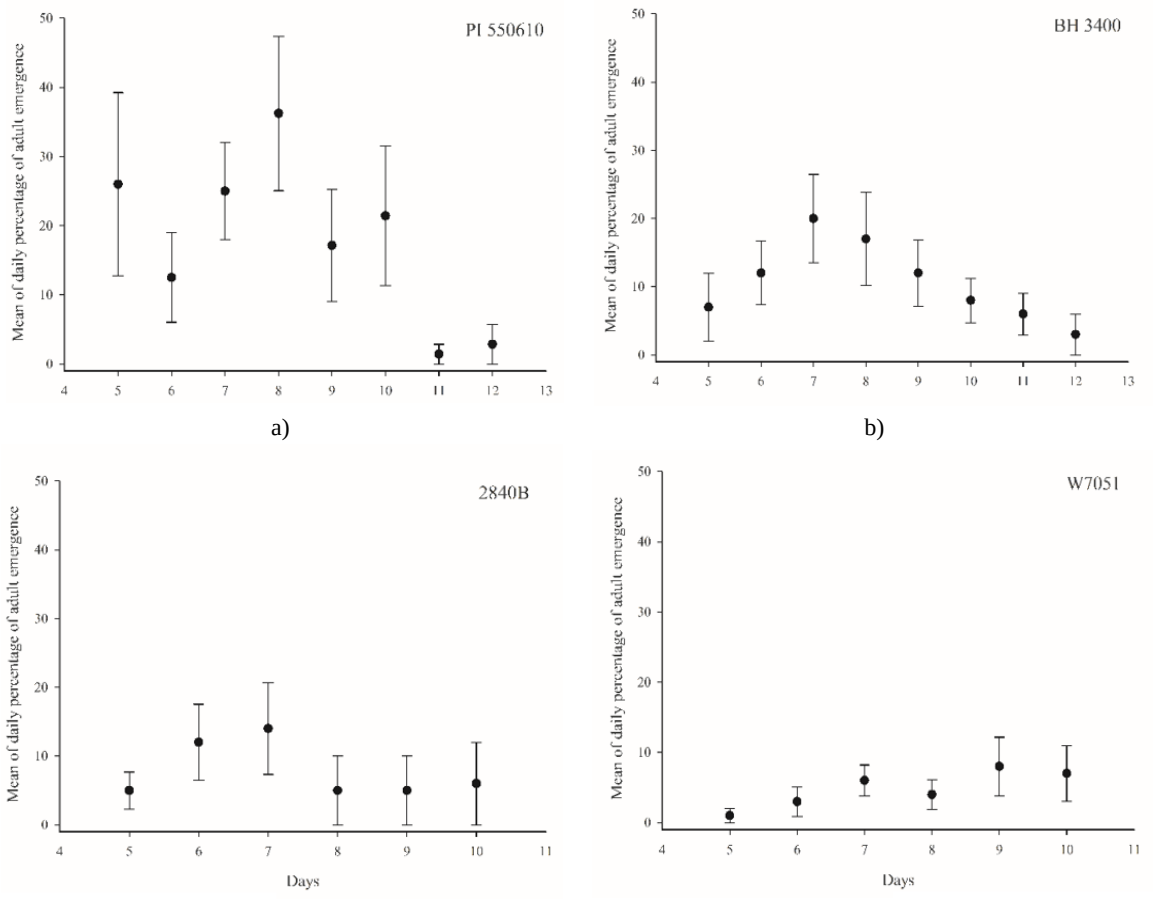
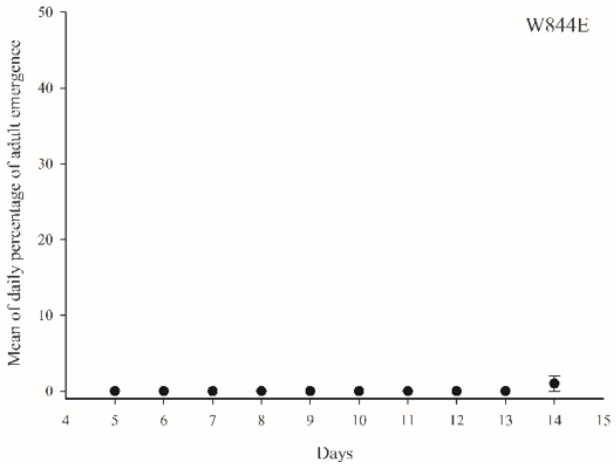


Figure 2. Nymphal survival (%) of *Melanaphis sacchari* in 12 sorghum genotypes under controlled conditions. Hays-KS, 2017.



a) b)

c) d)



e)

Figure 3. The mean daily (%) of the emergence of *Melanaphis sacchari* adults on five sorghum genotypes under controlled conditions. Hays-KS, 2017.

No difference was observed between treatments regarding the wax content on sorghum leaves, with means ranging from 0.0023 to 0.0054 g **(Figure 4)**. The M60GB31 genotype showed the highest wax content (0.0054 mg) and CHR 2042 the lowest (0.0023 g) **(Figure 4)**.

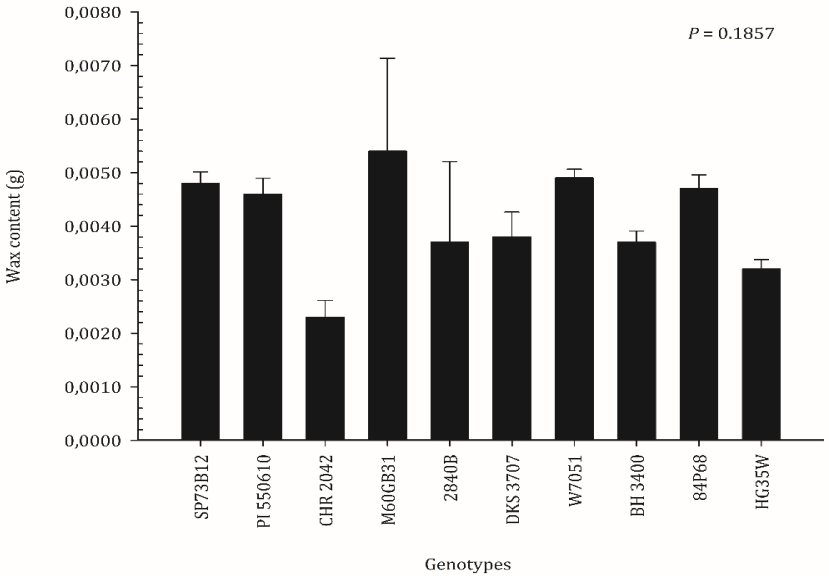


Figure 4. The mean (± EP) of the total content of epicuticular wax (g) extracted from five plants of ten sorghum genotypes. Hays-KS, 2017.

The low level of aphid colonization on a genotype suggests the presence of feeding and/or nymphoposition inhibitory factors, which may indicate the occurrence of plant resistance [20]. The results of this study indicated that in a no-choice test, the BH 3400, W7051, W844E, 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207 genotypes show a lower preference for nymphoposition and colonization by *M. sacchari*, indicating the possible expression of antixenosis and/or antibiosis resistance.

The colonization of aphids on host plants can be influenced by several chemical and morphological causes, as already reported for crops such as collard greens, where the content of glucosinolates, amount of wax on the leaf cuticle, and leaf hardness are determinant factors of resistance against *Brevycorine brassicae* (L.) (Hemiptera: Aphididae) [17]. In tomato plants, the presence of glandular trichomes and the high contents of acyl sugars or 2-tridecanone is directly related to the failure of *Myzus persicae*

(Sulzer) (Hemiptera: Aphididae) colonization [21], while in cotton, the gossypol content and the pilosity of the structures directly influences the infestation of *Aphys gossipii* (Glover) (Hemiptera: Aphididae) and feeding of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) in the different genotypes [22, 23].

In sorghum, several morphological and biochemical factors contribute to the difficulty of *M. sacchari* colonization, such as leaf quantity, small and narrow leaves, leaf curvature, greater distance between two leaves [24], presence of epicuticular wax [24] (morphological), nitrogen and chlorophyll levels [24], high p-hydroxybenzaldehyde contents (biochemical) and the genetic bases [25]. Although all these parameters have not been studied, some of these factors may be involved in the same genotype [26] which justifies the high nymphal mortality observed in this study.

Another determining factor that explains the high mortality of *M. sacchari* nymphs among genotypes is temperature [7, 27]. Growth chamber studies using different constant temperatures revealed that a cooler temperature interferes with the parameters of the biological development of *M. sacchari* and that the optimal temperature found for the development of the aphid is 28.3 °C, 5.3° above the temperature used in this study [27]. Even though it is found in higher latitudes, this aphid has also adapted to higher temperatures [27].

Similar studies under no-choice conditions conducted on *M. sacchari,* with only one resistant and susceptible sorghum genotype, showed that the data collected for the nymphal period in the resistant genotype (a strain of PI 550610) was like those found for PI 550610 (susceptible) in our study, 8 days approximately [28]. The similar data collected between the resistant genotype of those authors (3.09 nymphs) and PI 550610 (2.71 nymphs) in our study, was also found in the production of the number of nymphs per day. In another study, the PI 550610, being used as a resistant genotype, revealed that the percentage of nymphal survival and the total number of nymphs produced by *M. sacchari* was significantly higher than the susceptible genotype [18], as well as in our study. As the study of plant resistance to insects is a comparative study [16, 20], the data collected from the PI 550610 genotype showed that this genotype behaved as a susceptible material; however, there are works in which this genotype was classified as resistant.

The low nymphal survival ranging from 1 to 60% and the prolonged emergence of *M. sacharri* adults, ranging from 5 to 14 days, observed in PI 550610, 2840B, BH 3400, W7051, and W844E genotypes, may be a consequence of nutritional improprieties of the resistant plants during feeding purchased at the insects' nymphal stage [29]. This factor may also be the reason why the nymphs, that were conditioned to feed in BH 3400, W7051, W844E, 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207 genotypes, were not able to become adults. Genotypes carrying antibiosis resistance and those with pronounced antixenotic factors can cause deleterious effects on insect biology, especially in the early stages of development. This is due to the presence of secondary compounds present in sorghum seedlings whose deleterious effects require a longer period for the insect to complete the immature stage [30].

Epicuticular wax is a complex mixture of different aliphatic compounds, which contains compounds of alkanes, alcohols (primary and secondary), aldehydes, acids, ketones, β-dicetones, and esters [31, 32], whose proportions vary according to the genotypes and the environmental conditions in which the plant is developing. Although no difference was found between treatments regarding the wax content present in sorghum leaves, epicuticular wax can be deposited in different physical forms, such as thin layers, plaques, or crystals of different sizes and shapes [32]. These chemical-physical characteristics may confer the function of protection against the action of sucking pest insects during the feeding process [17]. It may also confer protection against the action of pathogens, excessive water loss, solar radiation, and the entry of chemicals and contaminants [33, 34].

CONCLUSION

All the genotypes evaluated in this study interfered with the biological parameters of *M. sacchari*. The associated occurrence of antixenosis and antibiosis resistance cannot be ruled out but requires more evaluation of the feeding behavior of the insect on the genotypes. Although all the study genotypes compromised

the biological performance of *M. sacchari*, the 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207 genotypes caused 100% nymphal mortality. The presence of chemical compounds (volatile or not) in the constitution of these genotypes may also be related to the possible expression of resistance in the genotypes evaluated. For future study, it is suggested that these compounds be identified and quantified with specific chemical analyses to isolate the two possible resistance mechanisms. These results are important for the species *M. sacchari* and may provide subsidies for future breeding programs focusing on resistance to aphids in the sorghum crop.

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CONFLICT OF INTEREST: None

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ETHICS STATEMENT: None

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