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Impact of Sorghum Genotypes on Nymphal Development and Reproduction of *Melanaphis sacchari*

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ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench), a tropical grass originating from Africa, serves as a staple food source for both humans and livestock. The global expansion of sorghum cultivation, particularly in Brazil and the United States, has facilitated the spread of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), a highly adaptable pest. This study evaluated adult fecundity and nymphal development of *M. sacchari* in 12 sorghum genotypes to identify potential sources of resistance, given the lack of environmentally friendly control strategies. A single sorghum seedling was used per replicate, each infested with 10 first-instar nymphs in a no-choice experiment, with ten replicates per genotype. Key parameters measured included the duration of the pre-reproductive and nymphal stages, total and daily nymph production, nymph viability, adult emergence rate, and leaf wax content. After 5 days of containment, the number of adults per plant was recorded. All genotypes negatively affected the biological performance of *M. sacchari*, with 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207 causing 100% nymphal mortality. No significant differences in leaf wax content were detected among genotypes. These findings provide valuable insights into the population dynamics of *M. sacchari* and provide a foundation for future breeding programs aimed at improving sorghum resistance to aphids.

Keywords: Sugarcane aphid, Host-plant resistance, *Sorghum bicolor*, Nymphoposition

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a tropical grass native to Africa, known for its diverse applications. It serves as a staple for human consumption and livestock feed, while also being used in the production of anhydrous alcohol, adhesives, alcoholic beverages, brooms, paints, and sugar. Additionally, due to its gluten-free properties, sorghum is gaining popularity in the food industry [1, 2].

For the 2021–2022 growing season, In Brazil, sorghum was cultivated across roughly 841.3 thousand hectares, yielding an estimated 2.4 million tons [3]. In the United States, sorghum ranks as the fourth most cultivated crop, following soybean (*Glycine max* (L.) Merrill), corn (*Zea mays* L.), and wheat (*Triticum aestivum* L.). The majority of U.S. sorghum production—around 65%—is concentrated in Texas and Kansas [4, 5].

Sorghum plays a key role in Brazilian agriculture due to its ability to thrive under challenging environmental conditions, including drought and low soil and air humidity. This adaptability makes it an essential crop for mitigating seasonal agricultural risks, particularly in the fall [6]. However, the expansion of sorghum cultivation has also facilitated the spread of *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), a polyphagous pest

that benefits from the increasing sorghum acreage in Brazil [3]. Sorghum fields provide a suitable habitat for *M. sacchari*, allowing both nymphs and adults to persist, ultimately leading to greater aphid populations and increased damage in subsequent growing seasons.

A major pest of sorghum in Africa, Asia, and Australia, *M. sacchari* was introduced to Brazil in the 1970s and has since become a significant concern [7]. While this aphid can infest sorghum seedlings immediately after emergence, the most severe outbreaks occur during dry periods [8]. Feeding on the abaxial leaf surface, *M. sacchari* causes visible plant damage, including purpling of seedlings, chlorosis, poor grain filling, delayed flowering, necrosis, and reductions in both yield and grain quality [7, 9]. Additionally, honeydew secretion fosters fungal colonization by *Capnodium* spp., which impairs plant respiration and photosynthesis [7]. Beyond direct feeding damage, *M. sacchari* is a vector for multiple viruses, including millet red leaf virus [10], sugarcane yellow leaf virus in both sorghum and sugarcane [11], and sugarcane mosaic virus (SMV) in sorghum [12].

Currently, the primary method for controlling *M. sacchari* in sorghum crops is the application of synthetic insecticides [9]. However, excessive pesticide use can have detrimental effects, such as environmental contamination, harm to non-target organisms, disruption of agroecosystem insect populations [13, 14], and the selection of resistant aphid strains, reducing the effectiveness of chemical control [15]. A promising alternative is the development of insect-resistant sorghum cultivars, which can help reduce pesticide dependence while complementing Integrated Pest Management (IPM) strategies. Plant resistance traits—including physical, chemical, and morphological factors—can influence aphid behavior and development, ultimately suppressing population growth and keeping infestations below economically damaging levels. This approach supports ecosystem conservation while enhancing agricultural profitability [16].

This study aims to assess the biological parameters of *M. sacchari* confined to 12 sorghum genotypes under controlled laboratory conditions. Given the increasing global significance of sorghum cultivation and the economic losses caused by *M. sacchari* and the viruses it transmits, research on aphid-plant interactions is essential for future food security and for improving sorghum production efficiency.

Materials and Methods

Experimental conditions

The study was conducted in climate-controlled chambers set to a temperature of 23 ± 1 °C, relative humidity of 65 ± 10%, and a photoperiod of 14 hours. The experiment took place at Kansas State University (KSU) in Hays, Kansas, United States (38º 51' N, 99º 20' W) during 2017.

Sugarcane aphid colony maintenance

A laboratory colony of *M. sacchari* was sustained on sorghum seedlings of a susceptible commercial hybrid (cv. P85Y40, Dupont-Pioneer, Johnston, IA) in a controlled environment with the same temperature, humidity, and light conditions as the experimental chambers. Sorghum seeds were sown in trays made of metal containing a soil mixture composed of peat moss, soil, and vermiculite in equal proportions (1:1:1). These seeds were germinated under controlled conditions, and the seedlings were watered day by day until they reached a height of 4.0 to 8.0 cm (three- to four-leaf stage). Aphid infestations were initiated at this stage. To ensure a continuous supply of aphids, fresh colonies were established weekly by transferring infested leaves onto new trays of sorghum seedlings. Only aphids from low-density populations were selected for experiments to minimize the risk of wing formation.

Bioassay setup

Each experimental unit consisted of a single sorghum seedling germinated in a 16.0-cm plastic cone (Stuewe & Sons, Corvallis, OR), which was filled with the same soil mixture used for aphid rearing. Three seeds were initially planted per cone, and after germination, only one healthy seedling was retained. Once the seedlings reached a height of 5.0 cm (two- to three-leaf stage, 5–6 days post-germination), they were each infested with 10 nymphs of *M. sacchari*. A total of 10 replicates were conducted for each treatment.

After infestation, each seedling was enclosed in a transparent plastic cylinder (30 cm in length) sealed with a plastic plug at the top. The sides of the cylinder had ventilation holes covered with fine mesh to allow airflow. All seedlings were secured in a supporting rack and placed inside the growth chamber, maintaining the same

temperature, humidity, and light cycle as before. The plants were watered every 48 hours by submerging the entire rack of cones in a water bath for 30 minutes.

Aphid performance assessment

Traditional methods for assessing aphid performance often involve isolating individual nymphs in clip cages and monitoring their fecundity by counting daily nymph production [17]. However, since many aphid species exhibit gregarious behavior, solitary development may impose stress, potentially leading to an underestimation of biological performance. In natural aphid colonies, group feeding provides advantages such as enhanced nutrient uptake (sink effects) [18]. Given the sugarcane aphid's natural tendency to aggregate, this study adopted a methodology that more accurately mimics field conditions by allowing nymphs to grow in groups rather than solo and in isolation.

The study recorded various biological parameters, including nymphal development duration, the pre-reproductive period, daily and total nymph production over 10 days, nymph survival rate, and adult emergence percentage. After 5 days of confinement, the number of adults present on the seedlings was recorded. Observations continued daily until the final nymph reached adulthood, at which point it was allowed to reproduce on the plant for ten days.

Wax analysis

To examine the epicuticular wax layer on both the abaxial and adaxial surfaces of sorghum leaves, 5 plants at the V3-V4 growth stage were selected from each treatment, with each plant serving as an independent replicate. The leaves were carefully detached using scissors, and each sample was separately immersed for 20 seconds in a 200 mL beaker containing 50 mL of pre-weighed chloroform. During this process, the beakers were gently agitated to facilitate wax dissolution. The resulting chloroform-wax solutions were left to evaporate in an exhaust hood until only solid wax residues remained. Once the solvent had completely evaporated, the beakers were reweighed, and the wax content was quantified by calculating the difference in mass before and after evaporation [17].

Statistical analysis

The collected data were analyzed using an analysis of variance (ANOVA), with normality and homogeneity of variances assessed through the Shapiro-Wilk and Levene's tests. When significant treatment effects were detected, mean comparisons were performed using Tukey's test ($P \leq 0.05$). All statistical analyses were conducted using the R statistical software, version 3.2.1 [19].

Results and Discussion

Out of the 12 sorghum genotypes tested, aphids placed on 5 of them (PI 550610, 2840B, BH 3400, W7051, and W844E) completed their nymphal development and reached adulthood **(Table 1)**. In contrast, the nymphs placed on the remaining 7 genotypes (84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207) failed to progress through the immature stages, resulting in 100 percent mortality **(Table 1)**.

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

Genotype	Nymphs/adult/d[1]	Nymphal period (d)[1]	Pre-reproductive period (d)[1]	Total of nymphs[1]
PI 550610	2.71 ± 0.45 (n = 6) a	7.92 ± 0.58 (n = 10) b	1.16 ± 0.28 (n = 6)	27.17 ± 4.52 (n = 6) a
2840B	2.17 ± 0.18 (n = 4) ab	7.37 ± 0.17 (n = 5) b	1.33 ± 0.22 (n = 4)	21.75 ± 1.75 (n = 4) ab
BH 3400	1.62 ± 0.15 (n = 9) ab	8.58 ± 0.47 (n = 9) ab	2.87 ± 0.44 (n = 9)	16.22 ± 1.54 (n = 9) b
W7051	1.18 ± 0.21 (n = 7) b	11.05 ± 1.42 (n = 8) a	3.28 ± 0.71 (n = 7)	11.85 ± 2.11 (n = 7) b
W844E	-	14.00 ± 2.00 (n = 1) a	-	-
84P68	-	-	-	-
CHR 2042	-	-	-	-
DKS 3707	-	-	-	-
HG35W	-	-	-	-

M60GB31	-	-	-	-
SP73B12	-	-	-	-
95207	-	-	-	-
P	0.0387	0.0478	0.0604	0.0387

Notes:
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.

No significant differences were found among treatments concerning the pre-reproductive period, with the duration ranging from 1.16 to 3.28 days **(Table 1)**. The genotypes W844E and W7051 (11.05 days) exhibited a notably longer nymphal period, showing statistical differences compared to the shorter periods observed in the 2840B and PI 550610 (7.92 days) genotypes, which had the fastest immature phase durations **(Table 1)**.

In terms of daily nymph production, the W7051 genotype recorded the lowest average, differing from PI 550610 (2.71 nymphs). For the W844E genotype, only one aphid reached the adult stage, and this individual did not produce any nymphs, making data collection for this parameter unfeasible.

Regarding the total nymph production over ten days, the W7051 and BH 3400 genotypes had the lowest totals, which were significantly lower than the production observed in PI 550610 **(Table 1)**.

The peak in nymph production for each genotype occurred between days 5 and 7 **(Figure 1)**. After this time, there was a decline in offspring production across all genotypes, continuing until the final day of evaluation at 10 days **(Figure 1)**.

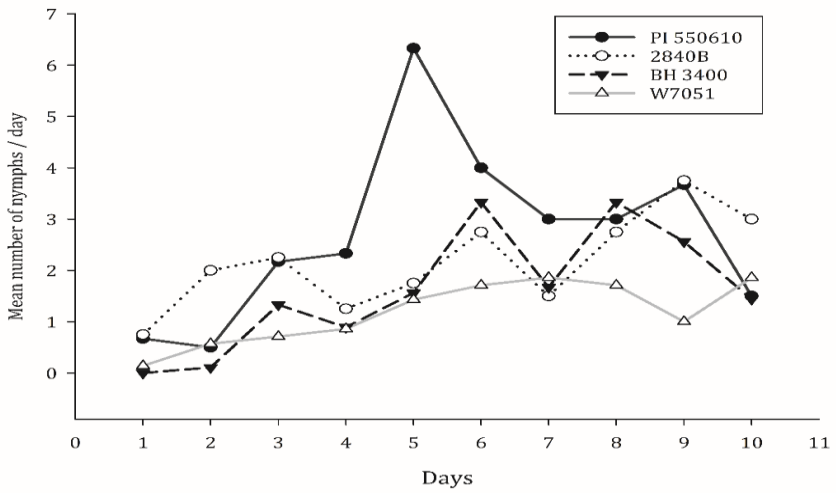


Figure 1. An average number of *M. sacchari* nymphs observed on four sorghum genotypes throughout the reproductive phase was recorded for each day of reproduction under controlled conditions (Hays-KS, 2017).

The survival rate of nymphs varied between 0% and 58%, with the genotypes 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, 95207 (all 0%), and W844E (1%) standing out **(Figure 2)**. The W844E genotype exhibited the lowest average day-by-day percentage of adult emergence, with 1% emergence observed only after 14 days **(Figure 3)**. In contrast, the W7051 genotype displayed adult emergence starting from day 5, reaching its peak emergence rate of 8% on day 10 **(Figure 3)**. Both the 2840B and BH 3400 genotypes displayed adult emergence from day 1, with their maximum emergence rates of 14% and 20% occurring on day 7 **(Figure 3)**. The PI 550610 genotype had the highest mean adult emergence at 36.25%, recorded on day 8 **(Figure 3)**.

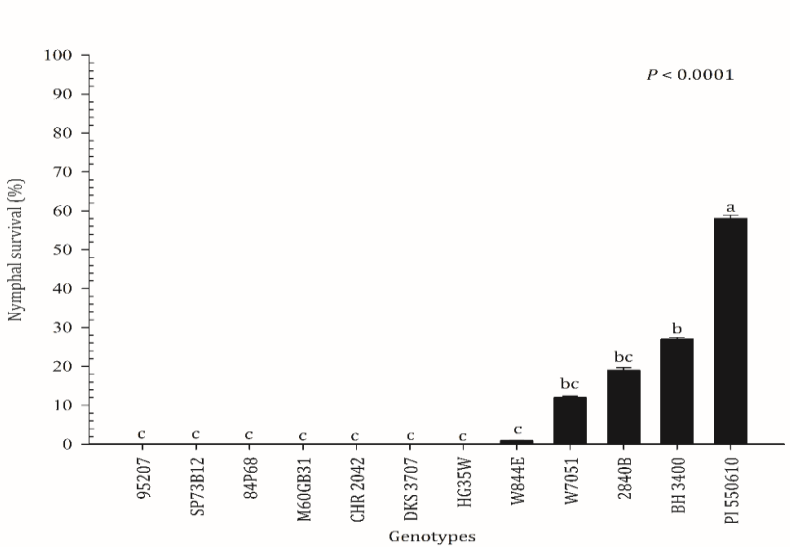
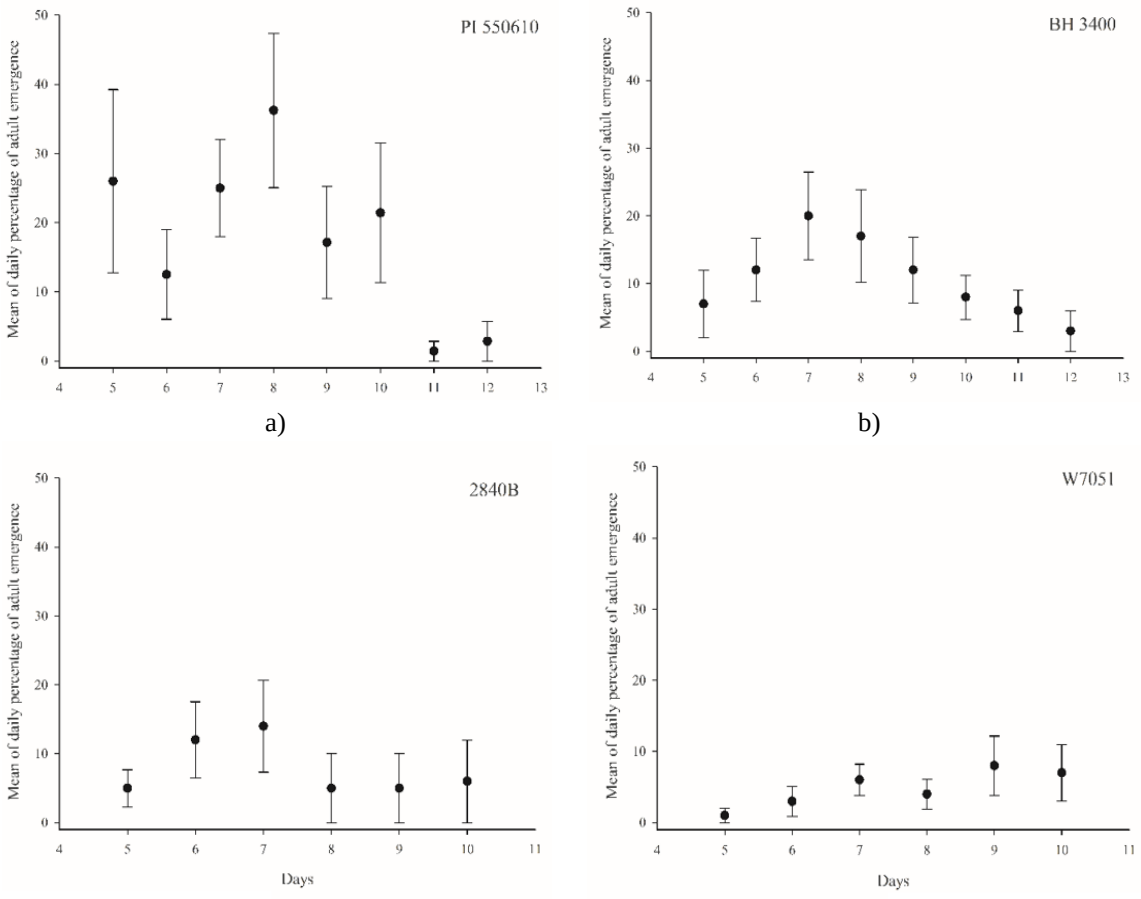
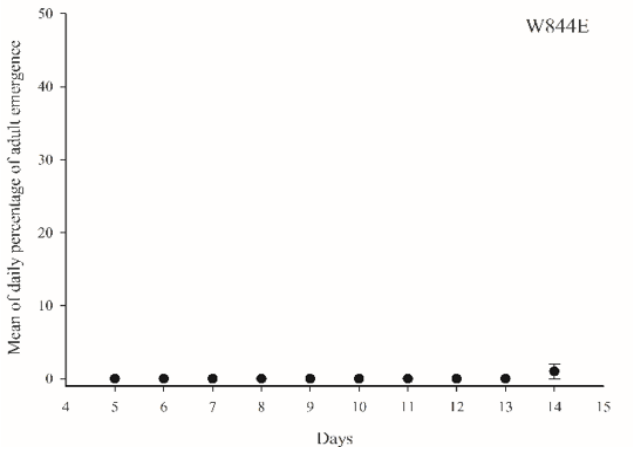


Figure 2. Percentage of *M. sacchari* nymph survival across 12 sorghum genotypes under controlled conditions (Hays-KS, 2017).





e)

Figure 3. Average daily percentage of adult *M. sacchari* emergence in five sorghum genotypes under controlled conditions (Hays-KS, 2017).

No significant differences were found in the wax content of sorghum leaves among the treatments, with values ranging from 0.0023 to 0.0054 g **(Figure 4).** The highest wax content (0.0054 g) was found in the M60GB31 genotype, while the lowest (0.0023 g) was observed in the CHR 2042 genotype **(Figure 4)**.

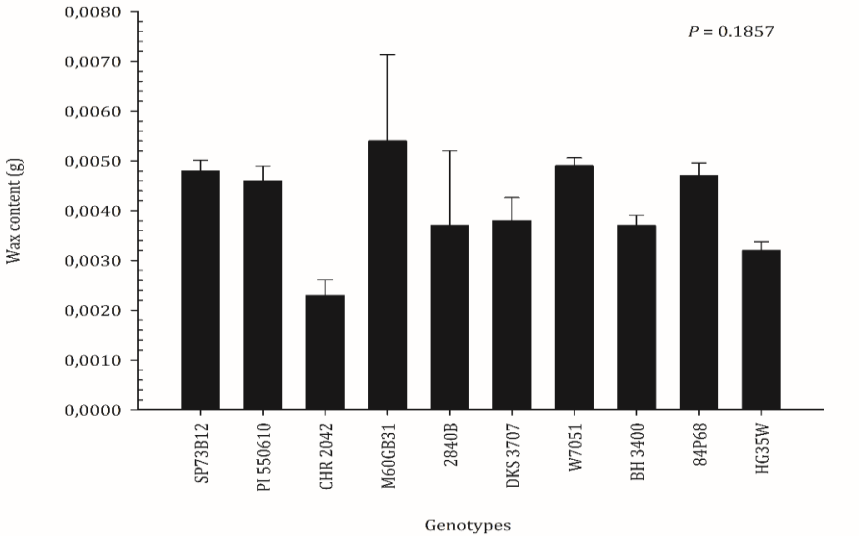


Figure 4. The mean (± EP) of the total content of epicuticular wax (g) extracted from five plants of ten sorghum genotypes (Hays-KS, 2017).

Low aphid colonization on certain genotypes suggests the presence of inhibitory factors affecting feeding and/or nymph production, possibly indicating resistance mechanisms in these plants [20]. The results from this study show that the genotypes BH 3400, W7051, W844E, 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207 exhibited lower aphid nymph production and colonization, which could reflect the presence of antibiosis resistance and/or antixenosis traits.

Several factors, both chemical and morphological, are known to affect aphid colonization on host plants. For example, in crops like collard greens, resistance to *Brevycorine brassicae* (L.) (Hemiptera: Aphididae) is linked to glucosinolate content, leaf wax levels, and leaf hardness [17]. Similarly, in tomatoes, the presence of glandular trichomes and high concentrations of acyl sugars or 2-tridecanone prevent colonization by *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) [21]. In cotton, the presence of gossypol and the pilosity of plant surfaces is critical in controlling infestations by *Aphis gossipii* (Glover) (Hemiptera: Aphididae) and feeding by *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) [22, 23].

For sorghum, factors influencing the difficulty of *M. sacchari* colonization include leaf size, narrowness, curvature, the spacing between leaves [24], the presence of epicuticular wax [24] (morphological factors), nitrogen and chlorophyll content [24], high p-hydroxybenzaldehyde levels (biochemical factors), and the plant's genetic makeup [25]. While not all of these variables were studied, they could contribute to resistance within a single genotype [26], which helps explain the observed high nymph mortality.

Temperature also plays a key role in the survival and development of aphids. Growth chamber experiments at various constant temperatures indicate that lower temperatures hinder the biological development of *M. sacchari*. The optimal temperature for aphid growth is 28.3 °C, which is 5.3 °C higher than the temperature used in the current study [27]. Despite being found in cooler latitudes, *M. sacchari* has adapted to thrive in higher temperature conditions [27].

In a similar study conducted under no-choice conditions, focusing on *M. sacchari* with one resistant and one susceptible sorghum genotype, the results from the resistant genotype (a strain of PI 550610) were comparable to those of the susceptible PI 550610 genotype in this study, with the nymphal period averaging around 8 days [28]. Furthermore, the number of nymphs produced per day in both the resistant genotype from the previous study (3.09 nymphs) and PI 550610 (2.71 nymphs) aligned closely with the findings in our work. In another investigation, PI 550610, considered a resistant genotype, demonstrated significantly higher nymph survival rates and greater nymph production compared to the susceptible genotype [18], a trend also observed in our study. Despite the data from this study indicating that PI 550610 behaves as a susceptible genotype, previous research has classified it as resistant, highlighting the complexity of plant resistance assessments [16, 20].

The observed low nymph survival (ranging from 1% to 60%) and delayed adult emergence (spanning from five to fourteen days) in the PI 550610, 2840B, BH 3400, W7051, and W844E genotypes may be attributed to nutritional deficiencies in the resistant plants during the nymph feeding stages [29]. This could also explain why the nymphs reared on BH 3400, W7051, W844E, 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207 genotypes failed to mature into adults. Genotypes with antibiosis resistance or strong antixenotic traits may negatively impact insect biology, particularly in the early development stages, as a result of secondary compounds in the plants. These compounds can extend the time required for the insect to finish its immature stage [30].

Epicuticular wax consists of a variety of aliphatic compounds, including alkanes, alcohols (both primary and secondary), β-diketones, acids, aldehydes, ketones, and esters [31, 32]. The composition and proportions of these compounds vary across different genotypes and environmental conditions. Although no significant differences were found in the wax content between treatments, the physical form of the epicuticular wax—whether as plaques, thin layers, or crystals—can vary. These physical characteristics may serve to protect the plant against damage from sucking insects, pathogens, excessive water loss, UV radiation, and the entry of chemicals and contaminants [33, 34].

Conclusion

The genotypes examined in this study had a significant impact on the biological development of *M. sacchari*. While the potential for antibiosis and antixenosis resistance is suggested, further investigation into the insect's feeding behavior on these genotypes is necessary to confirm this. Despite the overall effect of the genotypes on the aphid's biological performance, genotypes such as 84P68, CHR 2042, DKS 3707, HG35W, M60GB31, SP73B12, and 95207 exhibited complete nymphal mortality. The presence of certain chemical compounds, whether volatile or not, in these genotypes may be contributing to their potential resistance. Future research should focus on identifying and quantifying these compounds through detailed chemical analyses to better understand the mechanisms of resistance. These findings are valuable for understanding *M. sacchari* and could aid in the development of future breeding strategies aimed at enhancing aphid resistance in sorghum.

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