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# Multiple resistance to primary pests of grain sorghum hybrids: *Spodoptera frugiperda* (Lepidoptera: Noctuidae), *Diatraea saccharalis* (Lepidoptera: Crambidae), and *Diceraeus melacanthus* (Hemiptera: Pentatomidae)

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ABSTRACT

Plant resistance is an important tactic within the precepts of Integrated Pest Management, and the existence of grain sorghum hybrids with multiple insect resistance could benefit crop management and sustainability. This study evaluated the susceptibility of 30 grain-sorghum hybrids to three major pests, namely, fall armyworm (FAW) *Spodoptera frugiperda*, sugarcane borer (SCB) *Diatraea saccharalis*, and green-belly stink bug (GBS) *Diceraeus melacanthus*. The hybrids were cultivated and experiments with each insect species were conducted separately in a greenhouse. For FAW, visual injury assessments were performed on plants 7 and 14 days after infestation (DAI). For SCB, insect presence and injury were assessed 40 DAI. For GBS, the plants were evaluated using a damage rating scale 12, 19, and 26 DAI. Cluster analysis allowed separating the grain sorghum hybrids into groups regarding the levels of resistance to each pest. Hybrid BRS373 stood out as moderately resistant to FAW; AG1090, 80G20, BRAVO, BRS373, AG1615, and IG220 were the most promising for SCB; and for GBS, hybrids 50A40, A9735R, JADE, ENFORCER, BUSTER, 50A10, and IG244 were the most nominated. This information will significantly aid sorghum breeding programs focused on developing commercial hybrids that comprise both insect-resistance and high-yield characteristics. However, further research should evaluate potential chemical and morphological plant traits underlying the lower levels of susceptibility to FAW, SCB, and GBS found in the selected sorghum hybrids.

## Introduction

Sorghum, *Sorghum bicolor* (L.) Moench, is an important cereal crop used for grain, feed, and energy production. It is the fifth most produced cereal in the world, and can substitute corn in animal feed. Sorghum grains also constitute a staple food for people in some countries in Africa and Asia and hold promise for human consumption elsewhere due to their high levels of bioactive and functional compounds and gluten-free composition (FAO, 1995; Waquil et al., 2003; Martino et al., 2014).

In recent years, there has been a growing demand for sorghum-based products such as bread, pasta, cereal bars, snacks, cakes, biscuits, and beer, all of which show high added value due to the benefits for human nutrition and health as alternatives to versions with conventional cereals (Burdette et al., 2010; Abdelghafor et al., 2011; Martino et al., 2014; Cardoso et al., 2017). Brazil is currently the ninth largest sorghum producer worldwide, accounting for ~10% of total grain sorghum production. In the 2019 cropping season in Brazil, nearly 2.5 million tons of sorghum grains were produced, with the state of Goiás standing out among the Brazilian producing regions with more than 40% of the national production, followed by Minas Gerais state (IBGE, 2020). However, losses due to infestation of insect pests constitute one of the major problems in sorghum production. Insect pest attacks can occur in all phenological stages of sorghum, affecting the development and productivity of the plants. The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), has often been reported as one of the main pests of grain sorghum. It is one of the most harmful insect pests of annual crops in tropical regions of the Americas and is also an important pest of maize, cotton, soybean, and sugarcane, among other crops of economic importance (Cruz and Turpin, 1982; Boregas et al., 2013; Bernardi et al., 2015; Goergen et al.,

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2016). Thus, FAW infestations in these crops in adjacent areas or in succession can affect damage intensity in sorghum.

Another important lepidopteran pest is the sugarcane borer (SCB), *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae). The sugarcane borer makes galleries in the plant stems, weakening these structures and hampering photoassimilate translocation. In addition, SCB injures parts of the stem, causing indirect losses due to the entry of microorganisms.

Because of the cultivation of maize and sorghum in the second cropping season in succession with soybean, insect pests that were previously considered secondary have increased in frequency of occurrence in crops grown in no-tillage systems in Brazil, as is the case with the green-belly stink bug (GBS), *Diceraeus melacanthus* (Dallas) (Hemiptera: Pentatomidae). The green-belly stink bug has a habit of feeding and reproducing under the straw left by preceding crops, causing significant damage to maize and sorghum plants at the beginning of vegetative development, reducing vigor, and possibly causing the death of infested plants (Gassen, 1984, 1996; Corrêa-Ferreira and Sosa-Goméz, 2017; CONAB, 2022). When feeding, GBS typically positions itself at the base of the stem of host plants with the head facing downward and inserts the stylets into the stem to suck the phloem sap. Sap sucking is facilitated by injection of salivary enzymes that are toxic to the plant, hindering its development (Bianco, 2004; Grigolli et al., 2016).

In terms of the genetic variability of plants concerning insect attack, genotypes may have higher or lower levels of resistance/susceptibility (Bastos et al., 2015). Because the resistance of host plants to insects is horizontal in the majority of cases, i.e., resistance is controlled by various quantitative genes and loci, plants generally exhibit varying response levels to pest injury, as well as in terms of effects on insect feeding, oviposition, and development. Additionally, plant resistance can be specific for a species or group of insects, while the plant remains susceptible to other pests depending on the history of insect adaptation. Meanwhile, multiple resistance occurs when a plant genotype shows traits of resistance to various insect species (Kogan and Ortman, 1978; Lara, 1991; Baldin et al., 2019), which is a desired characteristic of commercial cultivars to be used in Integrated Pest Management (IPM) systems. Ultimately, host plant resistance is a control tactic that can be applied in combination with most control tactics within the IPM precepts to minimize problems caused by excessive use of chemical insecticides (Baldin et al., 2019).

Most studies that evaluated grain sorghum genotypes for resistance to SCB and other lepidopterans in Brazil were conducted over 20 years ago, especially during the 1980s and 1990s. In these studies, sources of resistance to SCB and FAW were found, to some extent, through the evaluation of plant parameters such as damage scores relative to larvae infestation, pest infestation intensity, and insect biology (Waquil et al., 1980, 2001; Boiça Júnior and Lara, 1983; Waquil and Santos, 1990; Lara et al., 1997). Because these evaluations were performed a long time ago, novel resistance screening phenotyping should be conducted with more recent and improved genotypes aiming at the introgression of resistance genes into germplasm of grain sorghum breeding programs. In addition, there is no information on the responses of sorghum genotypes in terms of resistance to insect pests such as GBS that have gained recent economic importance in agricultural systems.

Studying the behavioral and developmental parameters of insect pests and evaluating injury intensity on host plants is essential to help identify hybrids resistant to the main grain sorghum pests and to increase the management efficiency and profitability of producers (Knolhoff and Heckel, 2014). It also benefits breeding programs by allowing the production of hybrids that encompass desirable agricultural traits and moderate-to-high levels of resistance to major crop insect pests. Information available so far in the literature regarding potential multiple resistance of grain sorghum genotypes to SCB, FAW, and GBS is scarce, given that infestations of these pests in sorghum crops are relatively recent in the main producing regions. Thus, this study was conducted to evaluate the resistance of 30 grain-sorghum hybrids to three important pests of grain sorghum, namely, fall armyworm (FAW), sugarcane borer (SCB), and green-belly stink bug (GBS).

## Materials and methods

### *Experimental conditions and grain sorghum hybrids*

The study was carried out in a greenhouse at Embrapa Maize & Sorghum, in Sete Lagoas - MG, Brazil, under the conditions of 25 ± 5 °C, 70 ± 15% RH, and natural photoperiod. Thirty grain-sorghum hybrids already available on the market were evaluated for resistance to the three insect species (FAW, SBC, and GBS), namely, BRS373, FOX, DKB590, 1G220, AG1090, BRAVO, MSK327, 1G244, 50A10, 50A40, 50A70, 70G70, XGN1305, 80G20, MSK326, XB6022, AG1080, A9735R, AG304, 1G100, A9902, A9721R, AS1615, BUSTER, DKB550, MSK321, JADE, 1G232, ENFORCER, and MSK120.

Assays were conducted in three 20-L pots used for each evaluated hybrid, which contained soil fertilized with 50 g of NPK 08-28-16 and 0.3% of Zn/100 kg. Each pot was considered a replication, totaling 90 pots used in each experiment with different insect species. Thinning was performed 10 days after sowing, leaving three plants per pot. The assays with the three insect pests were carried out separately, in a completely randomized design.

### *Rearing of insect species*

The three insect species were reared in an air-conditioned chamber under controlled environmental conditions (25 ± 2 °C, 60 ± 10% RH, and 12 h light/12 h dark photoperiod).

**Fall armyworm (FAW):** test individuals of FAW were obtained from a rearing colony in the laboratory. Briefly, larvae were reared on an artificial diet based on cooked beans, wheat germ, and casein (Cruz, 2000). Adults were transferred to cylindrical PVC mating cages (40 cm height × 30 cm diameter) containing moth food (10% sugar and 5% ascorbic acid in water) and white sulfite paper on the inner walls for oviposition. The collected egg masses were left to hatch in plastic bags, and neonates were transferred to the artificial diet (Cruz, 2000). Newly hatched larvae obtained from the laboratory rearing colony were individually placed in 50-mL plastic cups sealed with acrylic lids (adapted from Mendes et al., 2011).

**Sugarcane borer (SCB):** adults of SCB were kept in PVC cages covered with sulfite paper for oviposition. The cages were covered with voile fabric on the top, which was fixed with rubber bands. The collected eggs were washed in a deionized water and 1% sodium hypochlorite solution, then in deionized water and in a solution of deionized water with 1% copper sulfate to avoid microorganism contamination. The eggs were dried at room temperature and subsequently placed in glass jars (8.5 × 2.5 cm) covered with voile fabric containing artificial diet based on soybean meal, sugar, and wheat germ [adapted from Hensley and Hammond (1968)], where they remained until the third instar (approximately 20 days old). Larvae were transferred to Petri dishes (5 cm diameter) with artificial diet cut into strips, due to the natural gallery-forming behavior of larvae. The larvae remained in the Petri dishes until adult emergence (Vilela et al., 2017).

**Green-belly stink bug (GBS):** GBS individuals were reared in plastic cages (37 × 12 cm) with perforated lids to allow for air exchange, pieces of voile fabric for oviposition, water-soaked cotton wool to provide water, and a mixture of seeds of common bean, sunflower, soybean, and peanut as the food source. The younger nymphal phases were kept separate from the adults.

*Fall armyworm assay*

For the assay with FAW, larval infestation was carried out when the sorghum plants were at the growth stage of four completely developed leaves. Five neonate larvae (<24 h old) were transferred per plant using a fine paintbrush. Three plants were placed in each pot, totaling 15 larvae per pot. The pots were covered with voile fabric to prevent the insects from escaping the plants.

Injury caused by FAW feeding on the plants was evaluated using a damage rating scale ranging from 0-9, according to that proposed by Davis for maize and adapted to sorghum (Davis and Williams, 1992): 0 = no visible injury; 1 = pinholes (more than one per plant); 2 = pinholes and 1 to 3 small circular lesions (<1.5 cm); 3 = 1 to 5 small circular lesions (<1.5 cm) and 1 to 3 elongated lesions (<1.5 cm); 4 = 1 to 5 small circular lesions <1.5 cm) and 1 to 3 elongated lesions (>1.5 cm and <3 cm); 5 = 1 to 3 large elongated lesions (>3 cm) in 1 to 2 leaves and 1 to 5 holes or elongated lesions (<1.5 cm); 6 = 1 to 3 large elongated lesions (>3 cm) in two or more leaves and 1 to 3 large holes (> 1.5 cm) on two or more leaves; 7 = 3 to 5 large elongated lesions (>3.5 cm) in two or more leaves and 1 to 3 large holes (>1.5 cm) on 2 or more leaves; 8 = many elongated lesions (>5 cm) of all sizes on most leaves and many medium-to-large holes (> 5) >3 cm on many leaves; and 9 = many leaves affected and almost totally destroyed. Injury scores were determined 7, 14, and 21 days after FAW larvae infestation (DAI).

*Sugarcane borer assay*

Upon showing four to six developed leaves (Magalhães et al., 2003), the sorghum plants were manually infested with five SCB neonate larvae per plant using a fine paintbrush. The neonate larvae (<24 h old) were obtained from the rearing colony.

Injury by SCB was evaluated 40 DAI on the sorghum plants. To this end, the plants were cut close to the ground and opened longitudinally with a razor blade to detect the presence of and quantify injury by SCB. The evaluated parameters were the number of healthy and bored internodes, length (cm) of the galleries made by SCB, and infestation intensity (%). Infestation intensity (I.I.) was calculated by the following formula: I.I. (%) = number of bored internodes/total number of internodes × 100 (Gallo et al., 2002).

*Green-belly stink bug assay*

For the assay with GBS, one adult stink bug was manually infested per plant, using one plant per pot. The adults were obtained from the rearing colony and were fasted for 16 h before infestation to stimulate feeding soon after being released onto the plant. Infestation was performed three days after seedling emergence, and one adult GBS was kept feeding on the plant for 12 days, according to the method of Roza-Gomes et al. (2011). During this period, all the pots were protected with perforated PET bottles to allow air circulation and prevent the insects from escaping. Evaluations on infested plants were carried out every two days to observe insect survival and replenish dead stink bugs. The first evaluation took place 12 DAI, soon after the removal of the insects from the plants.

Injury by GBS was evaluated based on the injury rating scale adopted by Roza-Gomes et al. (2011), as follows: score 0 = plants showing no injury; 1 = leaves with pinholes, no reduction in plant size; 2 = mild injuries on the plant whorl, which is partially twisted, with a reduction in size; 3 = unwrapped leaves or tillered plant; and 4 = dry or dead plant (Fig. 1). Nineteen days after infestation, i.e., seven days after the first evaluation, the second evaluation was performed, which


**Figure 1** Green-belly stink bug injury based on the damage rating scale adapted by Roza-Gomes et al. (2011) (0-4) to maize injury.

involved characterizing the injury score and the developmental stage of the plants, according to Roza-Gomes et al. (2011). Finally, 27 DAI, i.e., 14 days after the first evaluation, a third and final injury evaluation was performed on the plants.

### *Statistical analysis*

For univariate analysis, the data obtained in each bioassay for each insect species were subjected to the Shapiro-Wilk test to check for normality and to the Bartlett test to check for homogeneity of variances. Because the data did not follow a normal distribution or exhibit heterogeneity of variances, a nonparametric test was carried out using generalized linear model (GLM) and quasipoisson family, and the means of treatments were compared by Tukey's test ($\alpha$=0.05). Analyses were performed using R statistical software version 3.5.3 (R Core Team, 2014).

For multivariate analysis, the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) hierarchical clustering method was used in cluster dendrograms fixed with three groups and Euclidean distance was adopted as the dissimilarity unit measure. Analyses were performed using R statistical software version 3.5.3 (R Core Team, 2014).

## Results

### *Fall armyworm assay*

The first FAW injury evaluation on sorghum plants was carried out seven days after infestation (DAI), in which hybrid BRS373 showed a mean injury score lower than that of the other hybrids, followed by MSK327 ($F$= 2.10, df= 29, P< 0.007. These two hybrids maintained the lowest injury scores 14 DAI, together with hybrid A9735R ($F$= 1.94, df= 29, P< 0.01). Hybrids BRS373 and A9735R maintained the lowest injury scores 21 DAI ($F$= 2.22, df= 29, P< 0.004). Most of the hybrids showed lower injury scores 7 DAI than 14 and 21 DAI (Table 1).

By using the cluster dendrogram, it was possible to separate the grain sorghum hybrids into three groups according to their similarity regarding the evaluated plant injury parameters. Hybrid BRS373 was considered moderately resistant; MSK327 and A9735R were classified as susceptible; and the other hybrids were highly susceptible to FAW (Fig. 2).

**Table 1**
Mean (±SE) injury scores (1 to 9) caused by *Spodoptera frugiperda* larvae on grain sorghum hybrids at 7, 14, and 21 days after infestation (DAI).

| Hybrid | 7 DAI | 14 DAI | 21 DAI |
|---|---|---|---|
| **1G100** | 7.11 ± 0.11 a | 8.56 ± 0.44 a | 9.00 ± 0.00 a |
| **1G220** | 7.11 ± 0.11 a | 8.89 ± 0.11 a | 9.00 ± 0.00 a |
| **1G233** | 6.44 ± 0.29 a | 8.22 ± 0.22 a | 8.78 ± 0.11 a |
| **1G244** | 7.89 ± 0.29 a | 9.00 ± 0.00 a | 9.00 ± 0.00 a |
| **50A10** | 7.89 ± 0.40 a | 9.00 ± 0.00 a | 9.00 ± 0.00 a |
| **50A40** | 8.11 ± 0.11 a | 8.56 ± 0.44 a | 8.89 ± 0.11 a |
| **50A70** | 7.11 ± 0.68 a | 8.33 ± 0.67 a | 8.44 ± 0.56 a |
| **70G70** | 7.44 ± 0.29 a | 8.67 ± 0.19 a | 9.00 ± 0.00 a |
| **80G20** | 7.11 ± 0.40 a | 8.56 ± 0.22 a | 9.00 ± 0,00 a |
| **A6304** | 7.00 ± 0.19 a | 9.00 ± 0.00 a | 9,00 ± 0.00 a |
| **A9721R** | 6.89 ± 0.11 a | 8.22 ± 0.22 a | 9.00 ± 0.00 a |
| **A9735R** | 6.33 ± 0.69 a | 6.78 ± 1.90 ab | 7.00 ± 2.00 ab |
| **A9902** | 7.89 ± 0.48 a | 9.00 ± 0.00 a | 9.00 ± 0.00 a |
| **AG1080** | 7.33 ± 0.19 a | 8.22 ± 0.11 a | 9.00 ± 0.00 a |
| **AG1090** | 6.78 ± 0.22 a | 8.44 ± 0.29 a | 8.89 ± 0.11 a |
| **AS1615** | 7.67 ± 0.38 a | 8.44 ± 0.29 a | 9.00 ± 0.00 a |
| **BRAVO** | 7.11 ± 0.11 a | 8.44 ± 0.29 a | 8.89 ± 0.11 a |
| **BRS373** | 3.11 ± 1.57 b | 3.89 ± 1.95 b | 5.44 ± 2.00 b |
| **BUSTER** | 8.22 ± 0.11 a | 9.00 ± 0.00 a | 9.00 ± 0.00 a |
| **DKB550** | 7.89 ± 0.44 a | 8.78 ± 0.22 a | 9.00 ± 0.00 a |
| **DKB590** | 7.22 ± 0.22 a | 8.78 ± 0.22 a | 8.89 ± 0.11 a |
| **ENFORCER** | 7.56 ± 0.40 a | 8.78 ± 0.22 a | 9.00 ± 0.00 a |
| **JADE** | 7.78 ± 0.40 a | 9.00 ± 0.00 a | 9.00 ± 0.00 a |
| **MSK321** | 6.89 ± 0.48 a | 8.00 ± 0.33 a | 8.78 ± 0.22 a |
| **MSK326** | 7.11 ± 0.80 a | 8.89 ± 0.11 a | 9.00 ± 0.00 a |
| **MSK327** | 5.33 ± 1.95 ab | 6.67 ± 1.84 ab | 7.67 ± 1.33 a |
| **XB6022** | 7.67 ± 0.19 a | 9.00 ± 0.00 a | 9.00 ± 0.00 a |
| **XGN1305** | 7.22 ± 0.78 a | 8.67 ± 0.33 a | 9.00 ± 0.00 a |
| **MSK320** | 7.56 ± 0.56 a | 8.44 ± 0.56 a | 8.78 ± 0.22 a |
| **FOX ATLÂNTICA** | 7.11 ± 0.40 a | 8.89 ± 0.11 a | 9.00 ± 0.00 a |

*Means followed by different letters in the same column are different by Tukey's test (P < 0.05).*

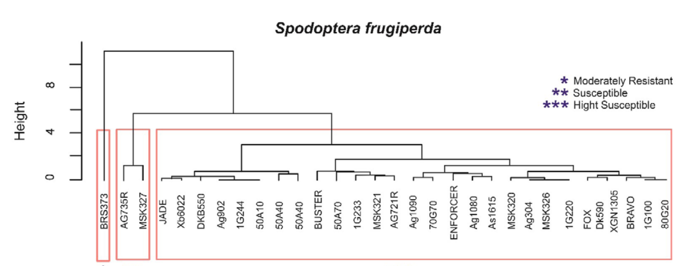


**Figure 2** Dendrogram of cluster analysis based on the Euclidean distance and grouping by UPGMA regarding scores of damage by *Spodoptera frugiperda* larvae on grain sorghum hybrids at 7 and 14 days after infestation.

### *Sugarcane borer assay*

In the assay with SCB, all parameters evaluated showed significant differences (P <0.05), except infestation intensity. In the assay with SCB, hybrids BUSTER and 50A70 had higher numbers of bored internodes ($F$= 8.21, df= 29, P< 0.0001) and, consequently, lower numbers of healthy internodes ($F$= 4.49, df= 29, P< 0.0001). In addition, no bored internodes were observed in hybrids AG1090, 80G20, AS1615, BRS373, BRAVO, and IG220, which consequently did not exhibit any injury (0% infestation intensity) ($F$= 2.12, df= 29, P< 0.006). Regarding the galleries caused by SCB feeding, hybrid 50A70 showed greater injury, followed by A6304, JADE, DKB590, and FOX ATLÂNTICA ($F$= 24.37, df= 29, P< 0.0001). Hybrids AG1090, 80G20, BRAVO, BRS373, AS1615, and 1G220 did not show any galleries (Table 2).

By using the cluster dendrogram, it was possible to separate the grain sorghum hybrids into three groups according to their similarity. Based on the evaluated parameters, hybrid 50A70 was classified as highly susceptible to SCB, while hybrids A6304, JADE, MSK321, BUSTER, FOX-ATLÂNTICA, XGN1305, DKB550, and DKB590 were considered susceptible. The other hybrids were classified as moderately resistant to SCB, among which AG1090, 80G20, BRAVO, BRS373, AS1615, and 1G220 stood out for showing no signs of injury on the plants (Fig. 3).

### *Green-belly stink bug assay*

In the assay with GBS, GLM analysis showed no significant difference between treatments on the different days of evaluation. However, we can highlight that hybrids 50A40 and A9735R showed the lowest values in the three evaluations, 12 DAI ($F$= 2.12, df= 29, P< 0.006), 19 DAI ($F$= 1.66, df= 29, P< 0.04), and 26 DAI ($F$= 1.87, df= 29, P< 0.02) (Table 3).

**Table 2**
Mean (±SE) numbers of healthy and bored internodes and gallery length (cm) caused by *Diatraea saccharalis* in grain sorghum hybrids. I.I = infestation intensity.

| Hybrid | Healthy internodes | Bored internodes | Galleries (cm) | I.I. (n.s) |
|---|---|---|---|---|
| **1G100** | 4.11 ± 0.44 bcd | 0.78 ± 0.29 ab | 2.89 ± 0.39 a | 13.36 ± 3.72 |
| **1G220** | 5.44 ± 0.78 cd | 0.00 ± 0.00 ab | 0.00 ± 0.00 abcde | 0.00 ± 0.00 |
| **1G233** | 4.33 ± 0.67 bcd | 0.22 ± 0.11 a | 4.33 ± 2.19 ab | 7.14 ± 4.96 |
| **1G244** | 3.89 ± 0.59 bcd | 0.44 ± 0.11 ab | 4.50 ± 1.26 ab | 11.11 ± 4.24 |
| **50A10** | 3.56 ± 0.40 abcd | 0.56 ± 0.11 ab | 4.00 ± 0.29 ab | 13.33 ± 3.26 |
| **50A40** | 3.11 ± 0.40 abcd | 0.78 ± 0.11 ab | 2.56 ± 0.06 a | 18.52 ± 1.85 |
| **50A70** | 1.00 ± 0.69 a | 1.56 ± 0.29 ab | 12.83 ± 0.05 e | 64.81 ± 20.87 |
| **70G70** | 3.56 ± 0.11 abcd | 0.44 ± 0.11 ab | 4.67 ± 0.88 ab | 13.33 ± 2.55 |
| **80G20** | 5.11 ± 0.80 bcd | 0.00 ± 0.00 ab | 0.00 ± 0.00 abcde | 0.00 ± 0.00 |
| **A6304** | 1.89 ± 0.29 ab | 1.00 ± 0.19 ab | 11.08 ± 0.74 de | 33.33 ± 6.42 |
| **A9721R** | 6.67 ± 0.58 d | 1.00 ± 0.33 ab | 4.14 ± 0.18 ab | 12.59 ± 2.63 |
| **A9735R** | 4.67 ± 0.58 bcd | 0.33 ± 0.00 a | 3.67 ± 0.33 ab | 5.29 ± 0.26 |
| **A9902** | 5.56 ± 0.29 cd | 1.00 ± 0.33 ab | 2.89 ± 0.11 a | 14.52 ± 4.73 |
| **AG1080** | 3.89 ± 0.29 bcd | 0.67 ± 0.00 ab | 4.08 ± 0.36 ab | 15.00 ± 0.00 |
| **AG1090** | 4.00 ± 0.51 bcd | 0.00 ± 0.00 ab | 0.00 ± 0.00 abcde | 0.00 ± 0.00 |
| **AS1615** | 5.33 ± 1.53 cd | 0.00 ± 0.00 ab | 0.00 ± 0.00 abcde | 0.00 ± 0.00 |
| **BRAVO** | 4.89 ± 0.40 bcd | 0.00 ± 0.00 ab | 0.00 ± 0.00 abcde | 0.00 ± 0.00 |
| **BRS373** | 3.44 ± 0.44 abcd | 0.00 ± 0.00 ab | 0.00 ± 0.00 abcde | 0.00 ± 0.00 |
| **BUSTER** | 4.00 ± 0.58 bcd | 1.78 ± 0.56 b | 4.74 ± 0.38 ab | 29.59 ± 8.56 |
| **DKB550** | 2.33 ± 0.19 abc | 0.78 ± 0.11 ab | 3.61 ± 0.45 ab | 24.07 ± 4.90 |
| **DKB590** | 4.00 ± 0.84 bcd | 0.89 ± 0.40 ab | 5.86 ± 0.58 abcd | 22.41 ± 10.20 |
| **ENFORCER** | 5.78 ± 0.87 cd | 0.89 ± 0.40ab | 3.42 ± 0.79 ab | 12.79 ± 6.75 |
| **JADE** | 2.89 ± 0.22 abc | 0.67 ± 0.00 ab | 8.83 ± 0.93 abc | 19.44 ± 1.60 |
| **MSK321** | 2.33 ± 0.38 abc | 1.22 ± 0.40 ab | 3.25 ± 0.43 cde | 37.96 ± 14.55 |
| **MSK326** | 2.89 ± 0.11 cd | 0.44 ± 0.11 a | 4.67 ± 0.67 ab | 14.81 ± 3.34 |
| **MSK327** | 4.00 ± 0.38 abc | 0.33 ± 0.00 ab | 2.50 ± 0.14 bcd | 7.22 ± 0.56 |
| **XB6022** | 5.11 ± 0.95 abc | 0.89 ± 0.22 ab | 4.44 ± 0.29 ab | 16.22 ± 4.65 |
| **XGN1305** | 3.56 ± 1.09 bcd | 0.89 ± 0.40 a | 3.33 ± 1.76 ab | 23.61 ± 13.68 |
| **MSK320** | 5.56 ± 0.68 bcd | 0.22 ± 0.11 ab | 7.08 ± 0.74 a | 4.07 ± 2.06 |
| **FOX ATLÂNTICA** | 2.89 ± 0.29 abcd | 1.22 ± 0.29 ab | 5.64 ± 0.07 ab | 28.52 ± 6.46 |

*Means followed by different letters in the same column are different by Tukey's test (P < 0.05). ns = not significant by Tukey's test (P< 0.05).*

**Table 3**
Mean (±SE) scores (1 to 4) of injury by adult stink bug (*Diceraeus melacanthus*) in grain sorghum hybrids at 12, 19, and 26 days after infestation (DAI).

| Hybrid | 12 DAI (ns) | 19 DAI (ns) | 26 DAI (ns) |
|---|---|---|---|
| **1G100** | 2.00 ± 0.33 | 2.33 ± 0.88 | 2.00 ± 1.00 |
| **1G220** | 2.33 ± 0.00 | 2.33 ± 0.33 | 2.00 ± 0.00 |
| **1G233** | 2.33 ± 0.33 | 2.00 ± 0.58 | 1.33 ± 0.33 |
| **1G244** | 1.33 ± 0.33 | 1.33 ± 0.33 | 1.33 ± 0.33 |
| **50A10** | 1.00 ±0.33 | 1.00 ± 0.00 | 1.00 ± 0.00 |
| **50A40** | 0.33 ± 0.33 | 0.33 ± 0.33 | 0.33 ± 0.33 |
| **50A70** | 2.33 ± 0.67 | 1.67 ± 0.33 | 1.33 ± 0.33 |
| **70G70** | 2.67 ± 0.67 | 2.33 ± 0.33 | 1.67 ± 0.67 |
| **80G20** | 3.67 ± 0.67 | 3.67 ± 0.33 | 3.67 ± 0.33 |
| **A6304** | 2.33 ± 0.33 | 2.33 ± 0.67 | 2.00 ± 0.58 |
| **A9721R** | 2.67 ± 0.33 | 1.67 ± 0.67 | 1.33 ± 0.33 |
| **A9735R** | 0.67 ± 0.67 | 0.33 ± 0.33 | 0.33 ± 0.33 |
| **A9902** | 2.33 ± 0.33 | 2.33 ± 0.33 | 1.33 ± 0.33 |
| **AG1080** | 2.67 ± 0.33 | 2.33 ± 0.33 | 2.00 ± 0.00 |
| **AG1090** | 1.67 ± 0.33 | 1.33 ± 0.33 | 1.33 ± 0.33 |
| **AS1615** | 1.33 ± 0.00 | 1.33 ± 0.33 | 1.33 ± 0.33 |
| **BRAVO** | 2.67 ± 0.58 | 2.33 ± 0.33 | 2.33 ± 0.33 |
| **BRS373** | 2.33 ± 0.33 | 2.67 ± 0.67 | 2.33 ± 0.88 |
| **BUSTER** | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 |
| **DKB550** | 2.00 ± 0.00 | 2.00 ± 0.58 | 1.00 ± 0.58 |
| **DKB590** | 2.33 ± 0.33 | 1.33 ± 0.33 | 1.33 ± 0.33 |
| **ENFORCER** | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 |
| **JADE** | 1.00 ± 0.33 | 1.00 ± 0.00 | 1.00 ± 0.00 |
| **MSK321** | 2.67 ± 0.33 | 2.67 ± 0.33 | 2.00 ± 0.58 |
| **MSK326** | 4.00 ± 0.33 | 3.67 ± 0.33 | 3.33 ± 0.33 |
| **MSK327** | 1.67 ± 0.33 | 1.67 ± 0.33 | 1.67 ± 0.33 |
| **XB6022** | 2.33 ± 0.33 | 1.67 ± 0.33 | 1.67 ± 0.33 |
| **XGN1305** | 2.33 ± 2.33 | 2.00 ± 0.00 | 2.00 ± 0.00 |
| **MSK320** | 2.33 ± 2.33 | 2.00 ± 0.00 | 2.00 ± 0.00 |
| **FOX ATLÂNTICA** | 1.33 ± 1.33 | 1.33 ± 0.33 | 1.00 ± 0.00 |

*ns = not significant by Tukey's test (P< 0.05).*

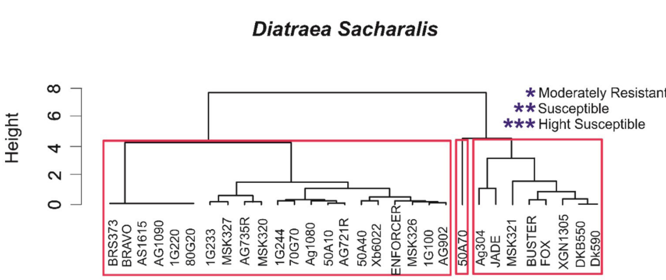


**Figure 3** Dendrogram of cluster analysis based on the Euclidean distance and grouping by UPGMA regarding the number of healthy and bored internodes, gallery length, and infestation intensity by *Diatraea saccharalis* in grain sorghum hybrids.

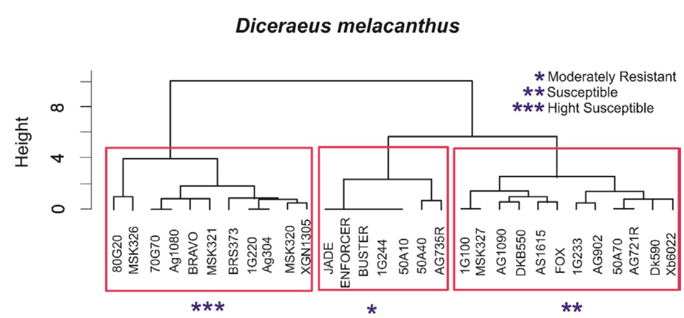


**Figure 4** Dendrogram of cluster analysis based on the Euclidean distance and grouping by UPGMA regarding scores of damage by *Diceraeus melacanthus* in grain sorghum hybrids at 12, 19, and 26 days after infestation.

These results corroborate multivariate analysis, which allowed separating the grain sorghum hybrids into three groups by using the cluster dendrogram according to their similarities for the evaluated parameters. Hybrids JADE, ENFORCER, BUSTER, IG244, 50A10, 50A40, and A9735R were classified as moderately resistant, the last two of which stood out with the lowest mean injury scores. Hybrids IG100, MSK327, AG1090, DKB550, AS1615, FOX-ATLÂNTICA, IG233, A9902, 50A70, A9721R, DKB590, and XB6022 were considered susceptible, whereas the other hybrids were classified as highly susceptible to GBS. Among these, 80G20 and MSk326 overall showed the highest GBS injury scores (Fig. 4).

## Discussion

The evaluation of hybrids revealed BRS373, MSK327, and A9735R as exhibiting the lowest injury by FAW. This injury evaluation methodology was also used and allowed for the differentiation of maize and sorghum genotypes in studies that applied the injury rating scale for FAW and complemented with a biological evaluation of pest development.

These studies found great variation in responses between genotypes, some of which were selected as potentially resistant, whether the evaluations were carried out in the field, greenhouse, or laboratory (Cortéz and Waquil, 1997; Gonçalves et al., 2011; Burtet et al., 2017; Crubelati-Mulati et al., 2019).

The presence of genes of resistance to FAW was highlighted in hybrids BRS373, MSK327, and A9735R. Differences regarding FAW injury between genotypes are not common, mainly due to the characteristics of high voracity and polyphagy of the larvae, which allow them to feed on various crop plants with a high degree of adaptability (Boregas et al.,2013). Therefore, the lower injury scores found here could be considered by sorghum producers as a reliable proxy for hybrid choice in terms of the potential for possessing some level of resistance to FAW attack.

Furthermore, although classified as highly susceptible to FAW, some hybrids such as 50A40, 50A70, A9902, AS1615, MSk321, MSk326, MSK327, XGN1305, and MSK320 did not show advances in injury between the two evaluations. This may be due to the resistance and/or tolerance levels of these plants. Because this study did not investigate insect parameters such as larval weight and mortality, but only injury to sorghum plants, minor injury may have occurred due to negative effects on insect feeding and growth (antixenosis- and antibiosis-resistance), as well as due to a better ability of the plants to support and regenerate damaged tissues (tolerance). However, it is not possible to ascertain these questions based on our assessments, so these evaluations should be carried out in the future to characterize the presence of resistance and/or tolerance.

Hybrids BRS373, BRAVO, AS1615, AG1090, 80G20, and IG220 were highlighted for not having SCB-made galleries, and hybrids MSK320, A9735R, IG233, and MSK327 for showing both the smallest-sized galleries and the lowest infestation intensity. Evaluations of gallery formation and infestation intensity are considered the most adequate parameters for identifying sources of resistance to SCB in sorghum (Waquil et al., 2001). Among those hybrids, BRAVO was also identified as one of the least susceptible to losses caused by *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in stored grains; this information is very important, given that this hybrid was also found to be less susceptible to the main pests throughout the whole crop cycle until the end of the production chain, i.e., from the field up to storage (Pimentel et al., 2018).

Infestation intensity is a more accurate plant parameter for predicting production losses, and this variable is also used in sugarcane and other types of sorghum as a proxy of levels of resistance to SCB attack (Milligan et al., 2003; Vilela et al., 2017; Araújo et al., 2019). According to Gallo et al. (2002), based on the infestation intensity and infestation percentage values, injury can be classified as low (0-5% and 0-25%); moderate (5-10% and 25-50%); regular (10-15% and 50-75%); high (15-25% and 75-95%); and extremely high (above 25% and 95%), respectively. For sugarcane, the economic threshold is fixed at 3% infestation intensity, with an infestation intensity of 1% possibly leading to reductions of up to 0.28% in alcohol production, 0.49% in sugar production, and 1.5% in stem production (Arrigoni, 2002). For sorghum plants, which are more tolerant to SCB attack in comparison to sugarcane, an economic threshold of 4% for infestation intensity can be used for decision-making in pest control (Vilela et al., 2014).

Studies evaluating different grain sorghum hybrids for resistance to SCB were performed some time ago, which included the evaluation of effects of fertilization, pest biology, and plant growth parameters (Lara, 1991; Cortéz and Waquil, 1997; Lara et al., 1997; López et al., 2000; Waquil et al., 2001; Bortoli et al., 2005). Results of these studies revealed correlations with ample genetic variability between sorghum genotypes, some of which were resistant to SCB. Of the genotypes that stood out for SCB resistance, hybrid AF-28 is known for possessing multiple insect resistance; in addition to SCB, the hybrid shows resistance to sorghum midge, *Stenodiplosis* (=*Contarinia*) *sorghicola* (Coquillet) (Diptera: Cecidomyiidae), and corn aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae). However, hybrid AF-28 exhibits undesirable agronomic characteristics, such as late development and non-erect formation (Rossetto et al., 1976; Boiça Júnior and Lara, 1983; Rossetto and Igue, 1983; Lara and Perussi, 1984; Lara et al., 1997). Therefore, evaluations of different genetic materials should not only take into account the resistance to the pest in question but should also consider other plant parameters, which can result in the identification of genotypes with all desired characteristics for cost-effective grain production.

The occurrence of GBS in maize and sorghum cropping systems has been frequent in recent years, due to the intensification of second crop cultivation soon after the harvesting of soybean (Corrêa-Ferreira and Sosa-Gómez, 2017). This is the first study conducted for the recommendation of grain sorghum hybrids with resistance to GBS. Here, plants of sorghum hybrids were infested with one adult stink bug, and hybrids 80G20 and MSK326 did not exhibit signs of recovery in the evaluations performed 19 and 26 DAI. Therefore, these were the hybrids most susceptible to GBS. Hybrids 50A40 and A9735R stood out as the most resistant to GBS, showing the lowest injury scores over the three evaluation dates (Table 3).

Studies carried out with maize genotypes using the injury scale for GBS are more common in the literature than with sorghum. Roza-Gomes et al. (2011) observed a mean injury score of 2.8 using the density of five stink bugs infested on five maize plants, starting from the V1 stage. In the present study, the use of the injury rating scale modified from maize evaluation was an efficient method to detect differences in susceptibility to GBS between the sorghum hybrids (Roza-Gomes et al., 2011; Duarte et al., 2015; Bridi et al., 2016; Cruz et al., 2016). Therefore, its use can be recommended in future studies evaluating resistance to GBS in grain sorghum.

Given that GBS is usually already present in the field at the beginning of the development of maize and sorghum plants, it is very important that the plants can recover from stink bug injury throughout their development; otherwise, they may die or show increased tillering, which is undesirable for grain sorghum production. Following stem and plant development, susceptibility to the pest is significantly reduced; however, when injury intensity is high, the chances of the plant recovering over time are lower. Therefore, the responses of the hybrids to GBS infestation should be evaluated early on in the first stages of plant development, as was done in this study, since it is the most susceptible growth stage when the insect is able to inflict damage (Sturza et al., 2020). In this way, in studies involving the selection of grain sorghum hybrids resistant to GBS, evaluations during these phenological stages can provide more precise and reliable results in terms of the presence of resistance and tolerance traits.

The 30 grain-sorghum hybrids evaluated here showed quite different responses to three major insect pests of the crop. This was expected, since host plant resistance is generally specific for a given pest species and the same genotype can be susceptible to other species. The same genotype may exhibit moderate resistance to another pest while also showing different results depending on environmental conditions due to gene × environment interactions. Additionally, other parameters can affect the responses of hybrids, such as phenological asynchrony, plant age, prior infestation by other pests or diseases, atmospheric conditions, soil fertility, amongst others (Rossetto et al., 1976; Baldin and Bentivenha, 2019).

In agricultural systems where FAW historically occurs in large populations, there is a need for more frequent monitoring to support decision-making regarding pest control measures such as application of chemical and biological insecticides at adequate times when the economic threshold is reached, although this information for FAW in sorghum is still lacking. Furthermore, given that the market value of sorghum grains has been high in the last years (CONAB, 2020), farmers have been concerned about plant health conditions, investing more time and resources in pest control measures (Rossetto et al., 1976; Boiça Júnior and Lara, 1983; Rossetto and Igue, 1983; Lara and Perussi, 1984; Cortéz and Waquil, 1997; Lara et al., 1997; Carvalho and Dias, 2020).

Despite the satisfactory results obtained here, where it was possible to indicate a less susceptible sorghum hybrid for each insect pest, in light of new techniques and prospects for research on host plant resistance, coupled with the increased crop importance in terms of planted area and economic profitability for sorghum farmers, more research with the aim of increasing productivity and resistance to the major insect pests is warranted. This is important, since sorghum is a plant species that host the three pests throughout its phenological development. The green-belly stink bug is a problem at the beginning of crop development, and is considered a limiting biotic stressor on plant growth at sensitive stages, to the extent that the greater the injury on the plants, the lower the chances of recovery (Fig. 5). The fall armyworm, in turn, can cause total leaf loss in the attacked plant, as was observed in this study in hybrids with high injury scores (8-9), totally undermining plant photosynthesis. Lastly, the sugarcane borer, which feeds on the stem, makes galleries that weaken the plants and may cause rotting by favoring the entry of microorganisms, which leads to mycotoxin accumulation, death of the apical meristem, reduction of sap flow, and general plant debilitation (Mendes et al., 2014; Silva et al., 2014) (Fig. 5).

As the main conclusions of this study in terms of the lower levels of susceptibility to attack by the three major insect pests, for improved management of FAW, hybrids BRS373, MSK327, and A9735R are recommended. For SCB, the highlighted hybrids are AG1090, 80G20, BRAVO, BRS373, AG1615, and IG220. Finally, according to cluster analysis and the numerical values of injuries, for GBS, hybrids 50A40, A9735R, JADE, ENFORCER, BUSTER, 50A10, and IG244 are the most promising. Further research should evaluate the potential chemical and morphological plant traits underlying the lower levels of susceptibility to FAW, SCB, and GBS found in the selected sorghum hybrids. This information will significantly aid sorghum breeding programs focused on developing commercial hybrids possessing both insect-resistance and high-yield characteristics.

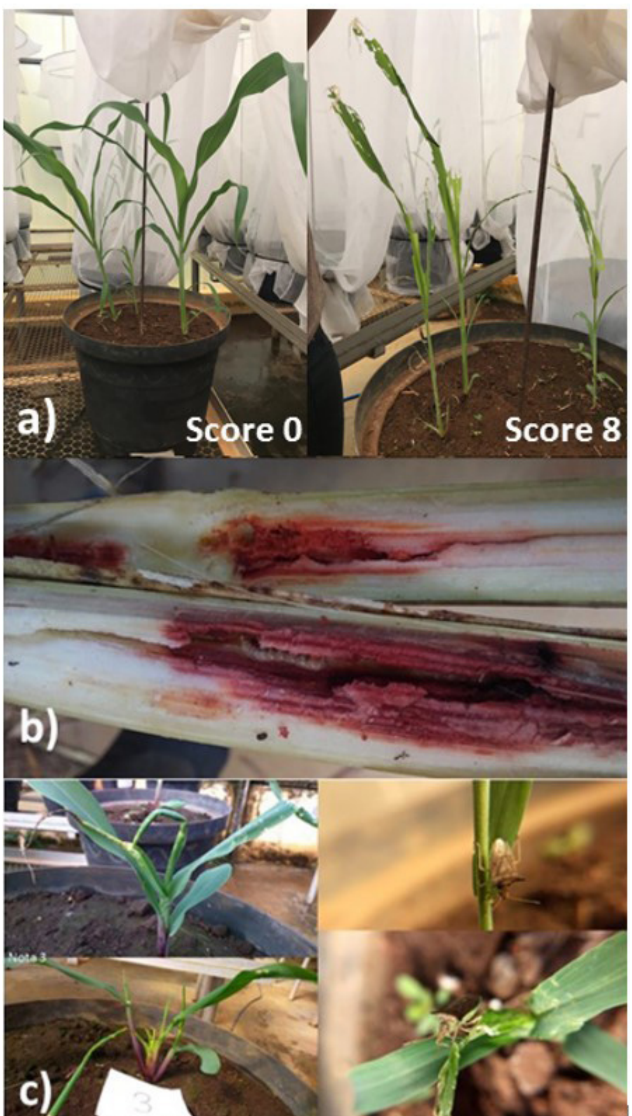


**Figure 5** Scores of injury on sorghum plants caused by *S. frugiperda* (a); *D. saccharalis* (b); and *D. melacanthus* (c).

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## Conflicts of interest

The authors declare no conflicts of interest.

## Author contribution statement

CSFS performed the methodology of the assays and wrote the manuscript; BHSS, PTN, MLFS and SMM helped with the writing of the manuscript; JCOF performed the statistical analysis of the data and created the graphs; and CBM donated the seeds of the hybrids to carry out the assay and helped with the writing of the manuscript.

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