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Original Article

Resistance of *bmr* energy sorghum hybrids to sugarcane borer and fall armyworm

Resistência de híbridos de sorgo energia *bmr* à broca-da-cana-de-açúcar e lagarta-do-cartucho

C. S. F. Souza[a], B. H. S. Souza[a], R. A. C. Parrella[b], M. L. F. Simeone[b], P. T. Nascimento[a], J. C. O. França[a], P. F. Lima[c], and S. M. Mendes[b]*

[a] Universidade Federal de Lavras, Campus Universitário, Departament of Entomology, Lavras, MG, Brasil
[b] Embrapa Milho e Sorgo, Sete Lagoas, MG, Brasil
[c] Universidade Federal de São João Del Rei, Campus de Sete Lagoas, Sete Lagoas, MG, Brasil

Abstract

The lower lignin content in plants species with energy potential results in easier cellulose breakdown, making glucose available for ethanol generation. However, higher lignin levels can increase resistance to insect attack. The objective of this work was to evaluate the susceptibility of a *bmr*-6 biomass sorghum (a mutant genotype with a lower concentration of lignin) to important pests of energy sorghum, *Diatraea saccharalis* and *Spodoptera frugiperda*. Experiments were performed in the laboratory and greenhouse to evaluate the development of these pests on the biomass sorghum *bmr* hybrids BR007, BR008, and TX635 and their respective conventional near-isogenic genotypes (without the *bmr* gene). The lignin content was higher in non-*bmr* hybrids, but the evaluated insect variables varied between treatments, not being consistent in just one hybrid or because it is *bmr* or not. The lowest survival of *S. frugiperda* was observed in the BR008 hybrid, both *bmr* and non-*bmr*. The *S. frugiperda* injury scores on plants in the greenhouse were high (>7) in all treatments. For *D. saccharalis*, there was no difference in larval survival in the laboratory, but in the greenhouse, the BR007 hybrid, both *bmr* and non-b*mr*, provided greater survival. Due the need to diversify the energy matrix and the fact that greater susceptibility of the *bmr* hybrids to either pests was not found in this study, these results hold promise for cultivation of these biomass sorghum hybrids for the production of biofuels.

Keywords: plant resistance, energy sorghum, *Diatraea saccharalis*, *Spodoptera frugiperda.*

Resumo

O menor teor de lignina em espécies de plantas com potencial energético resulta na maior facilidade de quebra da celulose, disponibilizando glicose para geração de etanol. Porém, maiores teores de lignina representa um fator de resistência ao ataque de insetos. O objetivo deste trabalho foi avaliar como importantes pragas do sorgo energia, *Diatraea saccharalis* e *Spodoptera frugiperda*, se comportam quanto à alimentação e desempenho em sorgo bmr-6, um genótipo mutante com menor concentração de lignina. Foram realizados experimentos em laboratório e casa de vegetação, avaliando o desenvolvimento destas pragas nos híbridos de sorgo biomassa *bmr* 007, 008, TX635 e seus respectivos genótipos isogênicos convencionais (sem o gene *bmr*). O teor de lignina foi maior nos híbridos não *bmr*, mas nos parâmetros avaliados nos insetos, houve variação entre os tratamentos, não sendo consistente em apenas um híbrido e nem por ser ou não *bmr*. A menor sobrevivência de *S. frugiperda* foi verificada no híbrido BR008 tanto *bmr* quanto não *bmr*. As notas de injúria por *S. frugiperda* no sorgo em casa de vegetação foram altas (>7) em todos os tratamentos. Para *D. saccharalis*, não houve diferença significativa para a sobrevivência larval em laboratório, mas em casa de vegetação o híbrido BR007 tanto *bmr* quanto não *bmr* proporcionaram maior sobrevivência. Diante da necessidade de diversificar a matriz energética e o fato de que não foi comprovada neste estudo maior suscetibilidade dos híbridos *bmr* a ambas as pragas, estes resultados são promissores para o cultivo desses híbridos de sorgo biomassa para produção de biocombustíveis.

Palavras-chave: resistência de plantas, sorgo energia, *Diatraea saccharalis*, *Spodoptera frugiperda*.

1. Introduction

One of the impediments to the conversion of biomass into biofuels is the presence of the polymer lignin, which interferes with the release of sugars from the cell wall polysaccharides cellulose and hemicellulose during enzymatic saccharification (Rubin, 2008; Dien et al., 2009). Despite the increase in the use of starch- and sugarcane-based biofuels, the fuels produced from lignocellulosic biomass are greenhouse-gas-favorable alternative energy

*e-mail: simone.mendes@embrapa.br
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sources (Rubin, 2008). Lignocellulosic biomass constitutes the residues of plants, such as elephant grass, coconut husk, and biomass sorghum, which do not have the sugar contents found in sugarcane and sweet sorghum (Santos et al., 2011; Hernández et al., 2015).

In Brazil, there has been a significant increase in the export of electricity from biomass in the last five years. The share of biomass sources out of the total composition of exported energy in the National Interconnected System (National Interconnected System) increased from 17% in 2013 to 19% in 2018 (EPE, 2019). This increase in the demand for heat generation from biomass is due to its lower cost and higher practicality, since it is used directly through combustion in ovens and boilers. Another important factor is that burning of fossil fuels emits various contaminants that cause local, regional, and global environmental impacts. In the Nationally Determined Contribution (NDC), Brazil is committed to reducing greenhouse gas emissions by 43% by 2030 and increasing the share of sustainable bioenergy in the energy matrix to approximately 18% by expanding its biofuels consumption, by increasing the share of advanced biofuels, known as second-generation biofuels (EPE, 2019).

Biomass sorghum (*Sorghum bicolor* L. Moench) is a promising lignocellulosic raw material because it is a productive crop adapted to water stress conditions, which is an advantage in a scenario that demands water savings, in addition to having a well-understood production system and being adapted to different environmental conditions (Silva et al., 2017). The biomass sorghum under suitable photoperiod conditions, has the potential to produce up to 102.22 t ha^{-1} of fresh biomass yield, its cultivation is completely mechanized, and the plants have calorific power similar to that of sugarcane, eucalyptus and elephant grass needed for burning, between 16 e 19 MJ·kg^{-1} (May, 2013; Parrella, 2013). Biomass sorghum can be used as a raw material for bioenergy through the production of second-generation ethanol as a liquid biofuel, and in energy generation by direct biomass burning, as well as food for ruminants (Cherney et al., 1991; Zegada-Lizarazu and Monti, 2012; Reddy and Blummel, 2020). The *brown midrib* (*bmr*) mutant of biomass sorghum is an alternative in the energy sector for use in processes in which lignin is an obstacle, such as the production of second-generation ethanol. The simplification of the saccharification process is very important because the cost of cellulases to degrade biomass is a limiting factor for the economic production of biofuels. For this, the raw material needs to have a low lignin content, as the biomass sorghum *bmr*, because cellulose is a glucose polymer that has to be broken down, so a lower lignin content means easier cellulose breakage, making glucose available for ethanol generation (Dien, et al., 2009). With high productivity and contribute to the strategy of a green economy with the supply of raw material in the distilleries, it is considered that biomass sorghum is a renewable and low-cost source of energy, being economically viable (Parrella, 2013; Vendruscolo et al., 2016). In addition, this mutant genotype may provide better digestibility to cattle because lignin is the nondigestible fraction of the plant that supports the stem. The higher the lignin content, the lower the silage quality and digestibility, as it is the factor that most limits the availability of cell wall components for bovine rumen microorganisms (Reddy and Blummel, 2020). The lignin cross-links cellullose and can be considered as the cell glue that gives resistance to plant tissue and gives rigidity to the cell wall, thus factors such as the incidence of pests can make the plant even more fragile (Rubin, 2008).

The occurrence of pest insects is a problem for sorghum crops, and the sugarcane borer *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) is one of the major pests. The greatest damage sugarcane borers can cause to a plant that can reach five meters in height is to make the stem fragile and susceptible to lodging, in addition to hindering the flow of sap and photoassimilates in the plant (Mendes et al., 2014; Silva et al., 2017). The fall armyworm, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), is also one of the main sorghum pests, causing defoliation and thereby reducing leaf area for photosynthesis. The presence of higher lignin content in plants can be a resistance trait to insect pests, especially lepidopterans (Dowd et al., 2016). Thus, the absence of this characteristic could reduce the natural morphological barrier of plants and increase their susceptibility to pest infestation. This possibility has not been fully elucidated in sorghum and results vary widely between studies (Dowd and Sattler, 2015; Dowd et al., 2016).

This study evaluated the susceptibility of *bmr*-6 sorghum to *D. saccharalis* and *S. frugiperda*, if the lower lignin content in the mutant plants favors feeding and pest performance. Knowledge of this information is highly important, since reducing the lignin content in the bioenergy feedstock could cause greater susceptibility to pest attack because this phenolic compound overall represents a plant defense trait against herbivorous insects (Dowd et al. 2016; Vendramim and Guzzo, 2009). Plants have chemical mechanisms for defense against insects, such as nitrogen compounds, terpenoids and phenolics. These compounds can be toxic to insects, preventing or altering their normal development (Vendramim et al., 2019). Lignin is made up of three main phenolic components: p-coumaryl alcohol (H), coniferyl alcohol (G) and synapyl alcohol (S), are aromatic polymers that vary in their branches and condense into different structures (Rubin, 2008). Therefore, the objective of this work was to evaluate the susceptibility of a *bmr*-6 biomass sorghum (a mutant genotype with a lower concentration of lignin) to important pests of energy sorghum, *Diatraea saccharalis* and *Spodoptera frugiperda*.

2. Material and Methods

2.1. Experimental site and conditions

Experiments with both pest species were carried out in a greenhouse and at the Laboratory of Ecotoxicology and Insect Management of Embrapa Milho e Sorgo located in Sete Lagoas, Minas Gerais state, Brazil, in a climate-controlled room with 25 ± 2 °C temperature, 12-hour photoperiod, and 60 ± 10% relative humidity.

2.2. Effect of the presence of the bmr gene on the development of sugarcane borer

The biomass sorghum *bmr* hybrids BR007, BR008, and TX635 and their near-isogenic genotypes (without the *bmr* gene) were evaluated in a laboratory bioassay with six genotypes (treatments). To obtain the leaves for feeding the insects, the hybrids were grown in the field, the soil in the region is of the red yellow latosol, with medium and silty texture (Embrapa, 2013). The design of the experiment was in randomized blocks, with four replications, each experimental plot consisting of three lines of 5 m in length and 0.7 m in spacing. The soil was fertilized with 400 kg ha^{-1} of NPK 8-28-16, and at 15 days after emergence, cover fertilization was performed using 200 kg ha^{-1} of urea. The plants were thinned 15 days after emergence, leaving eight plants per meter, in a total of 40 plants per row in plots. Management practices were performed according to May (2013), except that no insecticides were sprayed in the experimental area.

Testing insects were obtained from a rearing colony in the laboratory. Briefly, larvae were individually reared on artificial diet based on cooked beans, wheat germ, and casein (Bowling, 1967). Adults were transferred to cylindrical mating cages (40 cm h x 30 cm in diam.) containing moth food (10% sugar and 5% ascorbic acid in water) and white sulfite paper on the inner walls for oviposition. Collected egg masses were let to hatch, and neonates 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, according to the method adapted from Mendes et al. (2011) for *S. frugiperda*.

Whorl leaves from the *bmr* and non-*bmr* sorghum were collected from the plants when there were between six and eight fully developed leaves (stages V6-V8) (Magalhães and Durães, 2003) and taken to the laboratory, where they were cleaned and cut into pieces of approximately 50 cm^2. The leaves in the bioassay containers were replaced every 48 hours.

The survival and biomass of *D. saccharalis* larvae were evaluated 10 days after the beginning of the experiment. The evaluation was performed for 10 days, which is the duration of the behavior of *D. saccharalis* in seeking the plant stem; after that, the larvae no longer fed on the leaves. Data on these biological parameters were subjected to the Shapiro-Wilk and Bartlett tests to check the assumptions of normality of residuals and homoscedasticity, respectively. They were then subjected to analysis of variance (ANOVA), and means of treatments were compared by Tukey's test (α=0.05).

A second experiment was conducted in a greenhouse with the three biomass sorghum *bmr* hybrids (BR007, BR008, TX635) and their near-isogenic genotypes (without the *bmr* gene). The hybrids were planted in a completely randomized design to evaluate the resistance (antixenosis/antibiosis) to sugarcane borer. Planting was performed in 20 L pots filled with soil fertilized with 50 g of 08-28-16 NPK and 0.3% Zn/100 kg·v. For each treatment, 20 pots with three plants were used, and each pot was considered a replicate. At the four-to-six-developed leaf stage (Magalhães and Durães, 2003), the plants were infested with five newly hatched *D. saccharalis* larvae per plant, totaling 15 larvae per pot. Injury caused by bored larvae was evaluated every 60 days after infestation.

For injury evaluation, plants were cut close to the ground and opened longitudinally to detect the presence of galleries bored in the stem of plants. The parameters evaluated were plant height (cm), bored internodes (%), gallery size (cm), number of galleries per plant, survival (%) and biomass (mg) of larvae and pupae recovered from the plants. Data recorded for these parameters were subjected to the Shapiro-Wilk and Bartlett tests to check the assumptions of normality of residuals and homoscedasticity, respectively, and then analyzed by ANOVA. The means of treatments were compared by Tukey's test (α=0.05). The analyses were performed using the statistical R software version 3.5.3 (R Development Core Team, 2019).

2.3. Effect of the presence of the bmr gene on the development of fall armyworm

This experiment was carried out in the same manner as described for the sugarcane borer. The variables evaluated in *S. frugiperda* were larva-to-adult survival (%), biomass (mg) of larvae at 10 days, and biomass (mg) of pupae at 48 hours. To evaluate survival, a group of 10 individuals was considered one replicate, and there were nine replicates (90 individuals) in the experiment. For the other biological variables, one individual was considered a replicate. Because mortality was different in each treatment, the number of individuals (replicates) available for statistical analysis varied.

The adaptation index (AI) proposed by Boregas et al. (2013) was used to evaluate the larval performance of *S. frugiperda* whereby: AI= larval survival (%) × pupal biomass (mg)/larval development period (days); in the calculation of AI, pupal biomass was used to estimate the fecundity of adults (Barah and Sengupta, 1991). Correlation coefficients were also estimated to correlate the biological variables with the AI of *S. frugiperda*.

For the survival analysis, a curve was generated in SigmaPlot software 10.0® (Systat Software Inc., 2006) from the output of the chi-squared test. For the larval and pupal biomass data, the Shapiro-Wilk test was performed to check normality and the Bartlett test was used to check for homogeneity of variances. As data did not follow a normal distribution or exhibited heterogeneity of variances, was performed using generalized linear model (GLM) and negative binomial distribution, and the means of treatments were compared by Tukey's (α=0.05). The analyses were performed using the statistical R software version 3.5.3 (R Development Core Team, 2019).

The experiment to evaluate plant injury in the greenhouse followed the same procedure described for the sugarcane borer. Seven replicates were used per treatment, totaling 42 potted plants. Plants at the four-to-six-developed leaf stage were infested with five newly hatched *S. frugiperda* larvae per plant, totaling 15 larvae per pot. The pots were covered with a voile fabric cage (1.20 cm × 55 cm) to prevent the larvae from escaping.

Evaluation of plant injury was made through visual injury scores according to the scale proposed by Davis et al. (1992) for corn and adapted to sorghum. Evaluations were performed at 7, 14, and 21 days after larval infestation. The scores assigned to the plants ranged from 0 to 9, as follows: 0 = no injury; 1 = presence of pinholes (more than one pinhole per plant); 2 = pinholes and one to three small circular lesions (up to 1.5 cm); 3 = one to five small circular lesions (up to 1.5 cm), plus one to three elongated lesions (up to 1.5 cm); 4 = one to five small circular lesions (up to 1.5 cm), plus one to three elongated lesions (> 1.5 cm and < 3.0 cm); 5 = one to three large elongated lesions (>3 cm) in one to two leaves, plus one to five holes or elongated lesions (up to 1.5 cm); 6 = one to three large elongated lesions (>3 cm) in two or more leaves, plus one to three large holes (>1.5 cm) in two or more leaves; 7 = three to five large elongated lesions (> 3.5 cm) in two or more leaves, plus one to three large holes (greater than 1.5 cm) in two or more leaves; 8 = many elongated lesions (> 5 cm) of all sizes in most leaves, many medium to large holes (> five) larger than 3 cm in many leaves; 9 = almost completely destroyed leaves. The injury scores were analyzed by calculating their confidence intervals at 95% probability.

2.4. Bromatological analyses

Bromatological analyses were performed in the sorghum hybrids to identify possible differences in chemical composition between *bmr* and non-*bmr* genotypes. To determine the dry matter mass, the plants were placed in paper bags and dried in an oven at 65 °C for 72 hours. The samples were milled in a knife mill with a 2-mm sieve (Wiley mill, Arthur H. Thomas, Philadelphia, PA, USA) and prepared for chemical analysis.

The contents of acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) were determined according to the method proposed by Robertson and Van Soest (1981). The cellulose content was calculated as the difference between the ADF and lignin contents, and the hemicellulose content was calculated as the difference between NDF and ADF by near-infrared (NIR) spectroscopy (NIRFlex 500, Buchi Brasil Ltda, Valinhos, SP, Brazil). The calibration equations for the analysis of ADF, NDF, lignin, and calorific value were based on values obtained and stored in the Embrapa Corn and Sorghum database, which covers a total of 400 samples.

Data obtained from the chemical analyses were subjected to the Shapiro-Wilk and Bartlett tests to check the assumptions of normality of residuals and homoscedasticity, respectively, and were analyzed by ANOVA. The means of treatments were compared by Tukey's test (α=0.05). The analyses were performed using the statistical R software version 3.5.3 (R Development Core Team, 2019).

3. Results

3.1. Effect of the presence of the bmr gene on the development of sugarcane borer

There was no difference in larval survival of *D. saccharalis* among the energy sorghum hybrids. Sugarcane borer larvae fed the *bmr* hybrids showed mean 10-day-old biomass 22% higher than larvae reared in the non-*bmr* near-isogenic genotypes (Table 1).

The plant height, total number of internodes, number of healthy and bored internodes, and length and diameter of galleries, had no significant differences. The BR007 hybrid, both *bmr* and non-*bmr*, provided higher percentage survival of *D. saccharalis* than the other hybrids. The TX635 and BR007 hybrids had the highest and lowest mean pupal biomass, respectively (Table 2).

3.2. Effect of the presence of the bmr gene on the development of fall armyworm

There was difference in larval survival of *S. frugiperda* among the sorghum hybrids. TX635 had the highest percentage survival, followed by BR007 *bmr*, BR007, and TX635 *bmr*. The lowest larval survival was observed in the BR008 hybrid, both in the *bmr* genotype and conventional near-isogenic genotype (Figure 1).

Larval biomass differed between hybrids, with higher biomass in BR007 *bmr*, followed by TX635; the lowest biomass of fall armyworm was obsered in BR008, both *bmr* and non-*bmr*. The biomass of *S. frugiperda* pupae was greater in the BR008 hybrid than in the other sorghum hybrids (Figure 2).

Table 1. Means (± SE) of larval survival (%) and larval biomass (mg) at 10 days of *Diatraea saccharalis* in *bmr* and conventional non-*bmr* near-isogenic hybrids.

Hybrids	Survival	Biomass at 10 days
TX635	95.83 ± 3.14 a	12.22 ± 0.52 c
TX635 *bmr*	98.95 ± 1.04 a	19.77 ± 0.80 a
BR007	96.87 ± 2.19 a	14.71 ± 0.98 c
BR007 *bmr*	98.95 ± 1.04 a	15.40 ± 0.41 bc
BR008	93.74 ± 2.61 a	14.92 ± 1.08 c
BR008 *bmr*	98.95 ± 1.04 a	18.68 ± 0.98 ab

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

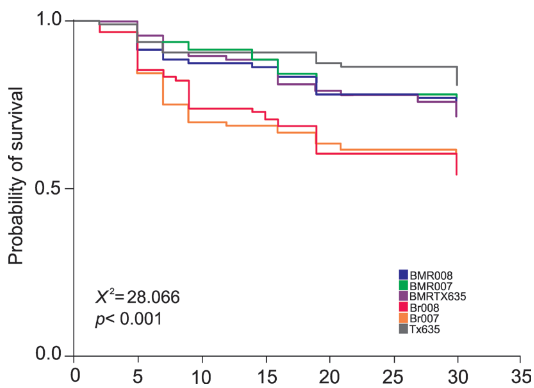

Figure 1. Survival curve of *Spodoptera frugiperda* as a function of days of development in *bmr* sorghum hybrids and their respective conventional non-*bmr* isogenic genotypes.

Table 2. Means (± SE) of plant height (cm), total number of internodes, number of healthy and bored internodes, gallery length and diameter (cm), and survival (%) and biomass (mg) of *Diatraea saccharalis* pupae in *bmr* sorghum hybrids and non-*bmr* near-isogenic genotypes.

VARIABLES	HYBRID	*Bmr*	non-*bmr*
	TX635	17.91 ± 2.42 a	19.31 ± 1.70 a
Plant height (cm)	BR007	22.09 ± 2.04 a	23.44 ± 2.22 a
	BR008	22.88 ± 1.93 a	22.97 ± 1.98 a
	TX635	4.43 ± 0.28 a	4.82 ± 0.25 a
Total internodes (nº)	BR007	5.23 ± 0.31 a	5.42 ± 0.44 a
	BR008	5.20 ± 0.36 a	5.22 ± 0.32 a
	TX635	3.43 ± 0.29 a	3.78 ± 0.25 a
Healthy internodes (nº)	BR007	4.00 ± 0.26 a	4.43 ± 0.35 a
	BR008	3.93 ± 0.32 a	3.98 ± 0.32 a
	TX635	1.00 ± 0.28 a	1.03 ± 0.25 a
Bored internodes (nº)	BR007	1.23 ± 0.31 a	0.98 ± 0.44 a
	BR008	1.27 ± 0.36 a	1.23 ± 0.32 a
	TX635	2.77 ± 0.60 a	2.60 ± 0.49 a
Gallery length (cm)	BR007	2.66 ± 0.48 a	1.88 ± 0.48 a
	BR008	2.67 ± 0.59 a	2.89 ± 0.52 a
	TX635	0.27 ± 0.04 a	0.38 ± 0.09 a
Gallery diameter (cm)	BR007	0.29 ± 0.04 a	0.32 ± 0.04 a
	BR008	0.33 ± 0.04 a	0.31 ± 0.04 a
	TX635	52.78 ± 0.88 b	51.67 ± 0.79 b
Survival (%)	BR007	58.75 ± 2.08 a	59.12 ± 1.43 a
	BR008	51.25 ± 1.08 b	49.86 ± 1.04 b
Pupal biomass (mg)	TX635	133.73 ± 1.58 ab	135.67 ± 2.26 a
	BR007	125.55 ± 3.18 ab	111.04 ± 2.49 c
	BR008	123.99 ± 3.28 b	131.94 ± 2.74 ab

Means followed by different letters in the same column differ according to Tukey's test (P < 0.05).

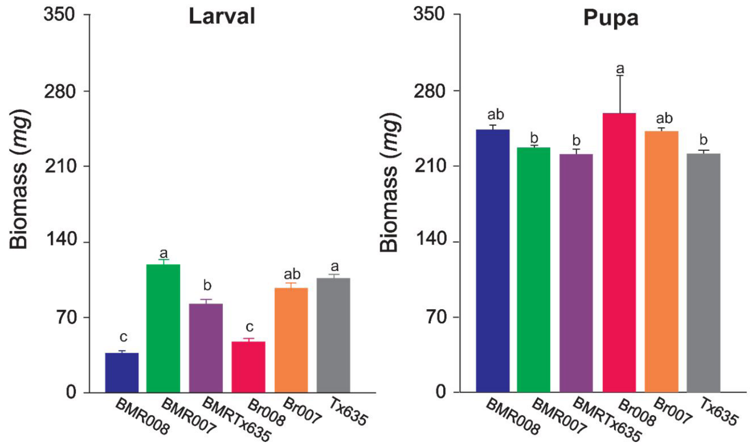

Figure 2. Response of *Spodoptera frugiperda* feeding on leaves of *bmr* sorghum hybrids and their respective isogenic genotypes: larval biomass (mg) and pupal biomass (mg). Data are means and standard errors. Means followed by different letters differ according to Tukey's test (P < 0.05).

The AI of *S. frugiperda* was 11.64 in the TX635 hybrid, 10.08 in TX635 *bmr*, 11.36 in BR007, 10.45 in BR007 *bmr*, 10.01 in BR008, and 9.07 in BR008 *bmr*, without difference between means. The estimated correlation coefficient between the AI and larval survival was 0.875; between AI and pupal biomass, -0.390; and between AI and larval biomass, -0.877.

The injury scores of *S. frugiperda* in sorghum plants differed among treatments. The TX635 hybrid, both *bmr* and non-*bmr*, had a lower injury score at 7 days than at 14 or 21 days after infestation, as did the BR007 *bmr* hybrid. The other hybrids did not differ across the evaluated days. The highest injury scores were found in the TX635 *bmr* and non-*bmr* hybrids, with the highest score (9) observed in the last evaluation date (Figure 3).

3.4. Bromatological analyses

The levels (%) of ADF, NDF, and hemicellulose had no differences among the energy sorghum hybrids. The dry matter was highest in the BR007 non-*bmr* hybrid and lowest in the TX635 non-*bmr* hybrid. Lignin percentage was higher in the non-*bmr* hybrids than in the conventional near-isogenic genotypes. Finally, the calorific value (MJ/kg) was highest in the BR008 non-*bmr* hybrid and lowest in TX635 *bmr* (Table 3).

4. Discussion

The hypothesis of greater susceptibility to attack of sugarcane borer and fall armyworm due to the lower lignin content in *bmr*-6 sorghum hybrids was not supported by our findings. Although microorganisms that can degrade cellulose, hemicellulose, and lignin have been identified in the midgut of *D. saccharalis* larvae, they have a greater capacity to digest cellulose than hemicellulose, and few produce enzymes to degrade lignin (Dantur et al., 2015). Thus, plant genotypes with lower lignin levels, in theory, could be more consumed by insect pests because lignin is difficult to digest. Our results showed that *D. saccharalis* larval biomass was higher in *bmr* than in non-*bmr* sorghum hybrids. This finding demonstrates the effect of plant biomass on insect development, as the greater the biomass, the greater the insect growth rate is, indicating that the host plant is suitable for herbivore development and does not show resistance (Souza et al., 2019). It is possible that the *bmr* hybrids are more suitable for the development of *D. saccharalis*, which harbors microorganisms capable of digesting lignin, though our survival data do not show such a trend.

The height, total number of internodes, number of healthy and bored internodes, and length and diameter of galleries made by *D. saccharalis* did not show significant differences between *bmr* and non-*bmr* genotypes or among sorghum hybrids. The BR007 hybrid, both *bmr* and non-*bmr*, caused higher *D. saccharalis* percentage survival than the other hybrids; TX635 provided greater pupal biomass; and the non-*bmr* BR007 hybrid, lower pupal biomass. Thus, there was no consistency in the results that would show an effect of the *bmr* gene on *D. saccharalis*. Again, the results of the greenhouse experiment did not indicate higher levels of resistance in the energy sorghum *bmr* genotypes relative to the non-*bmr* genotypes.

Higher survival of *S. frugiperda* larvae was found in the TX635 hybrid than in the respective near-isogenic *bmr* genotype under laboratory conditions. This hybrid had a greater difference in lignin content between the *bmr* and non-*bmr* genotypes, and there may also be other causes of resistance involved besides this trait, which are still unknown. The leaves of the *bmr* genotypes had lower lignin contents, as shown by the results of the bromatological analysis, which was expected. Lower lignin contents may make plants more susceptible to herbivory, since lignin is

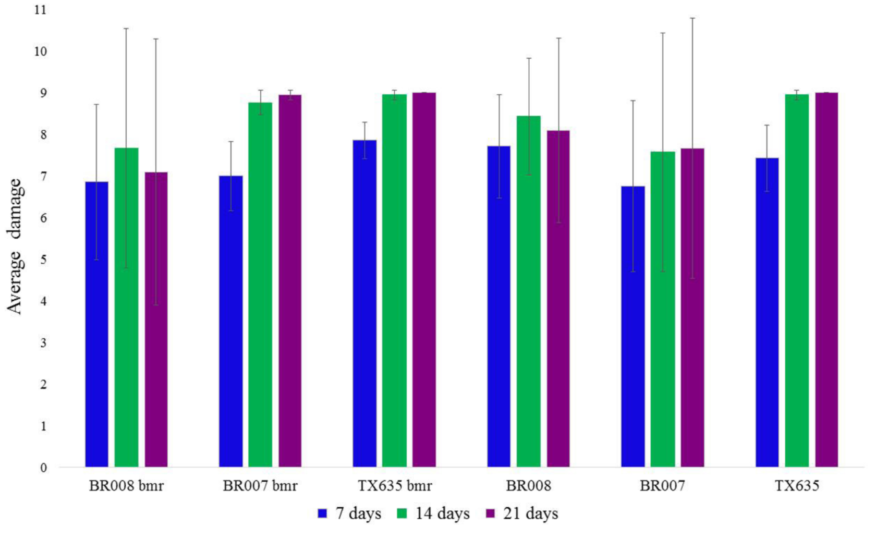

Figure 3. Injury scale (0-9) for *Spodoptera frugiperda* at 7, 14, and 21 days after infestation by recently hatched larvae in the different hybrids of *bmr* sorghum and their respective conventional isogenic genotypes. Intervals between adjacent bars do not differ from one another by the confidence interval (P < 0.05).

Table 3. Means (± SE) of variables of the bromatological analysis of *bmr* and non-*bmr* sorghum hybrids.

VARIABLE	HYBRID	*Bmr*	non-*bmr*
Dry matter 65 °C (MG ha^{-1})	TX635	14.23 ± 0.03 ab	11.48 ± 0.80 b
	BR007	14.13 ± 0.46 ab	17.42 ± 2.11 a
	BR008	12.87 ± 1.25 ab	15.05 ± 0.93 ab
ADF (%)	TX635	35.28 ± 0.91 a	37.92 ± 0.96 a
	BR007	33.88 ± 2.13 a	36.65 ± 3.06 a
	BR008	34.74 ± 2.20 a	35.46 ± 3.35 a
NDF (%)	TX635	58.93 ± 0.92 a	63.96 ± 1.42 a
	BR007	58.60 ± 2.50 a	60.89 ± 3.69 a
	BR008	59.86 ± 0.30 a	60.50 ± 3.12 a
Lignin (%)	TX635	3.24 ± 0.08 c	4.79 ± 0.20 a
	BR007	3.21 ± 0.07 c	3.89 ± 0.12 b
	BR008	3.26 ± 0.18 c	4.64 ± 0.37 a
Hemicellulose (%)	TX635	24.75 ± 0.53 a	26.04 ± 0.92
	BR007	25.27 ± 0.74 a	24.69 ± 0.79
	BR008	25.54 ± 0.41 a	25.78 ± 0.74
Calorific value (MJ/kg)	TX635	16.49 ± 0.08 b	16.71 ± 0.17 ab
	BR007	16.73 ± 0.23 ab	16.61 ± 0.14 ab
	BR008	16.71 ± 0.22 ab	17.03 ± 0.05 a

Means followed by different letters in the same column differ according to Tukey's test (P < 0.05).

an important chemical and morphological component of plant resistance that can hinder larval feeding (Dowd et al., 2016). However, the BR008 hybrid, both *bmr* and non-*bmr*, caused lower larval survival and biomass of *S. frugiperda*, which did not differ. Thus, the presence or absence of the *bmr* gene did not alter the larval performance. These results indicate that *S. frugiperda* larvae reached the adult stage in a similar manner in all energy sorghum hybrids. The same occurred for the pupal biomass, as there was no difference of whether the hybrids were *bmr* or not, and the BR008 hybrid provided greater pupal biomass.

Pencoe and Martin (1982) found a significant positive correlation between pupal biomass and adult fertility in *S. frugiperda*. Data obtained in the present study suggest equality in the biology of *S. frugiperda* when fed a *bmr* vs. a non-*bmr* sorghum genotype. Therefore, apparently reducing the lignin content and changing the biomass composition of plants for bioenergy production does not necessarily increase the susceptibility of sorghum to *S. frugiperda* attack, and this process would contribute to the sustainable production of biofuels.

The *S. frugiperda* biological variables were more affected by the effect of hybrid than the *bmr* mutation; the *bmr* genotypes did not negatively affect the insect development. The BR008 hybrid was the one that most negatively affected the biology of *S. frugiperda*, given the observed results of higher larval mortality and growth inhibition. This suggests that *S. frugiperda* may be functionally susceptible to this hybrid, which warrants further investigation.

The AI of *S. frugiperda* varied from 9.07 in the BR008 *bmr* hybrid to 11.64 in TX635. Boregas et al. (2013), in a study evaluating the AI of *S. frugiperda* in different host plants, considered values above 10 high because this is the value found in maize plants, which is the main host of fall armyworm. The results of the present study demonstrated that the AIs were above 10 in all treatments, except for BR008 *bmr* (9.07), that was still very close to 10. This reinforces the hypothesis that the *bmr* mutation does not affect *S. frugiperda* development. In another study, Ribeiro et al. (2020) compared the effect of forage species on the development of *S. frugiperda* and found AI of 26.49 for maize, and 22.02 for *Cynodon dactylon* plants, which was the species more similar to maize. Both values were higher than those found in the present study; nevertheless, *S. frugiperda* developed well in the evaluated energy sorghum hybrids.

Dowd et al. (2016) also did not find consistency in the survival data of *S. frugiperda* in *bmr* sorghum leaves. Those authors evaluated fall armyworm survival for more than one harvest and observed a greater effect of harvest than of the *bmr* mutation. Additionally, Dowd and Sattler (2015) found no differences in *S. frugiperda* mortality in *bmr* sorghum. The authors suggested the presence of the brown midrib in leaves of *bmr* sorghum may, contrary to expectations, increase the resistance of the plants, since they may be less nutritionally suitable for larvae because of this trait and possibly because of other chemical and morphological changes related to the presence of the *bmr* mutation. Our results also suggest this conclusion because although no microorganism that can digest lignin was found in the midgut of *S. frugiperda* (Dantur et al., 2015), the *bmr* mutation had no effect on fall armyworm.

Regarding the *S. frugiperda* injury scores in sorghum hybrids in the greenhouse, no differences were observed between the *bmr* and non-*bmr* genotypes. The highest injury scores were found in the TX635 *bmr* and non-*bmr* hybrids, with maximum scores (9) observed at 14 and 21 days of larval infestation. The hybrids studied herein had the *bmr*-6 mutation, which causes reduced cinnamyl alcohol dehydrogenase activity (Oliver et al., 2005). When evaluating *bmr*-6 and *bmr*-12 sorghum hybrids, Dowd et al. (2016) did not detect consistent susceptibility to *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) or *S. frugiperda* in any *bmr* genotype compared to the susceptibility of the nonmutant isogenic genotypes. However, those authors reported evidence of increased resistance in the *bmr*-6 genotypes compared to the near-isogenic genotypes, and greater susceptibility of the *bmr*-12 plants to the insects both in the field and in laboratory. The results obtained by Dowd et al. (2016) differ from the results found herein, as we did not consistently find increased resistance or susceptibility of the *bmr* gene to the sugarcane borer and fall armyworm. This subject is still not resolved given the varying responses found in the literature.

Plant dry matter was highest in the BR007 non-*bmr* hybrid and lowest in the TX635 non-*bmr* hybrid. Thus, the BR007 genotype was more productive than the *bmr* genotype, which may contribute to a higher tolerance to pest infestation relative to the other two hybrids. Conversely, the calorific value was highest in the BR008 non-*bmr* hybrid and lowest in TX635 *bmr*. This trait of higher energy content is important for energy generated from direct burning of biomass (Parrella, 2013).

Cellulose is the major structural component of plant cell walls; hemicellulose is the second most abundant component in lignocellulosic biomass; and lignin is the compound that gives greater rigidity to plant fibers and confers resistance to insects and pathogens. However, this trait hinders biofuel production (Dien et al., 2009; Rubin, 2008; Rio et al., 2007; Van Wyk, 2001). Although a reduction in lignin concentration was detected in the *bmr* genotypes, there were no differences in ADF, NDF, or hemicellulose contents, which did not differ between the *bmr* and non-*bmr* sorghum hybrids. Ebling and Kung Junior (2004) also found no differences in the percentage of NDF or ADF between *bmr* and non-*bmr* corn, but found differences in their lignin content. These findings corroborate the results found herein, where the percentage of lignin was higher in non-*bmr* sorghum hybrids; the TX635 hybrid had the highest lignin content; and the greatest difference in lignin content was found between the *bmr* and non-*bmr* genotypes.

The parameters evaluated for *D. saccharalis* and *S. frugiperda* in our study varied between treatments, and were not consistent or predominant in only one energy sorghum hybrid or in *bmr* or non-*bmr* genotypes. From the viewpoint of integrated pest management, it is interesting that there is resistance to pests in non-*bmr* sorghum that is grown for other purposes than biomass, such as energy cogeneration through direct burning. However, for biofuel production, which is the main purpose of *bmr* sorghum plants due to its lower lignin content, the fact that it has no greater susceptibility to the main crop pests is highly important.

We can conclude that cultivation of *bmr* energy sorghum can be safe within the context of integrated pest management because it is not more susceptible to the major crop pests, sugarcane borer and fall armyworm, and regardless of whether the sorghum is *bmr* or not, control measures, using chemical and biological approaches should be applied whenever the economic thresholds are attained. Given the need to diversify the energy matrix in Brazil and worldwide since renewable energy is a key source of energy security, provides reduced dependence on fossil fuels, and causes to lesser emission of greenhouse gases, the results of this work show that cultivation of these biomass sorghum hybrids holds great promise for biofuel production.

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