



CAMILA DA SILVA FERNANDES SOUZA

**SORGHUM (*Sorghum bicolor*): A STUDY OF THE RESISTANCE
TO CROP PESTS IN BRAZIL**

**LAVRAS – MG
2021**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutor.

Prof. Dr. Bruno Henrique Sardinha de Souza
Orientador

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SORGO (*Sorghum bicolor*): UM ESTUDO DA RESISTÊNCIA À INSETOS-PRAGA NO BRASIL

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A minha filha Lara, meu maior amor.

*Ao meu marido Thiago e aos meus pais Ronaldo (in memorian) e Wania pelo amor, cuidado,
carinho e apoio incondicional.*

Dedico

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RESUMO

As interações inseto-planta envolvem mecanismos de sobrevivência, seleção genes de resistência de plantas e quebra dessa resistência por insetos. De tais interações resultam o desenvolvimento de mecanismos de defesa nas plantas os quais fazem pressão de seleção sobre os insetos, caracterizando processos coevolutivos. Esta tese reuniu três artigos com a cultura do sorgo visando a resistência às principais pragas. No primeiro artigo o objetivo foi avaliar a suscetibilidade do sorgo *bmr*-6 um genótipo mutante com menor concentração de lignina às pragas *Diatraea saccharalis* e *Spodoptera frugiperda*. 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*. 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 híbridos. 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. O segundo artigo teve como objetivo avaliar a influência da infestação *D. saccharalis* sobre a produção de grãos em híbridos comerciais. Para isso, híbridos de sorgo granífero foram plantados em três safras: março de 2018 (2018/1) (SAFRA 1), setembro de 2018 (2018/2) (SAFRA 2) e março de 2019 (2019/1) (SAFRA 3). No experimento, foram utilizados seis tratamentos, sendo os três híbridos de sorgo granífero e os mesmos três híbridos com tratamento químico com o produto Altacor® (150 ml ha⁻¹), sob infestação natural, em quatro repetições. Os parâmetros avaliados foram: comprimento das galerias (cm), altura da planta (cm), comprimento (cm) e peso (g) das panículas, intensidade da infestação e produtividade. A intensidade de infestação das três safras foi maior quando não tratada com inseticida e resultou em menor produtividade. Em relação à diferença entre as safras, o plantio da 1^a safra apresentou menor intensidade de infestação. O híbrido DKB 590 apresentou o menor nível de tolerância, sendo que com inseticida a produção aumentou 1,5 vezes na safra 1 (149,77%). Na safra 2, o híbrido BRS 373 apresentou o menor aumento na produtividade de grãos com controle (29,79%), seguido pelo AG1090 com 33,13%, e novamente DKB 590 (40,79%) com maior dependência de controle químico para aumentar a produtividade. A safra 3 obteve o menor rendimento entre as três safras, e o híbrido AG1090 respondeu melhor ao controle químico com aumento de 105,90% no rendimento, seguido pelo DKB 590 com 54,26% e BRS373 com 38,19%. O terceiro artigo avaliou a suscetibilidade de 30 híbridos de sorgo granífero a *S. frugiperda*, *D. saccharalis* e *Diceraeus melacanthus* em casa-de-vegetação. Para a lagarta-do-cartucho, a avaliação nas plantas foi realizada 7 e 14 dias após a infestação (DAI). Para a *D. saccharalis* em 40 DAI e para o percevejo barriga-verde aos 12, 19 e 26 dias após a infestação. Por meio da análise de agrupamento, foi possível separar os híbridos de sorgo granífero em grupos quanto aos níveis de resistência a cada praga. O híbrido BRS373 se destacou como moderadamente resistente a *S. frugiperda*; os AG1090, 80G20, BRAVO, BRS373, AG1615 e IG220 foram os mais promissores para *D. saccharalis*; e para *D. melacanthus*, os híbridos 50A40, A9735R, JADE, ENFORCER, BUSTER, 50A10 e IG244 foram os mais indicados. Conclui-se que o sorgo é suscetível as três espécies aqui estudadas, a seleção de híbridos é específica para cada espécie, o menor teor de lignina no sorgo não deixa a planta mais suscetível a *D. saccharalis* e *S. frugiperda*, e o sorgo granífero pode ser muito sensível ao ataque de *D. saccharalis*, diminuindo a produtividade em até 1,5 vezes dependendo do híbrido, época de plantio, falta de monitoramento e controle adequado.

Palavras-chave: Resistência de plantas. *Spodoptera frugiperda*. *Diatraea saccharalis*. *Diceraeus melacanthus*. Sorgo. Granífero. Sorgo energia.

ABSTRACT

The insect-plant interactions involve survival mechanisms, selection of plant resistance genes and the breaking of this resistance by insects. These interactions result in the development of defense mechanisms in plants which exert selection pressure on insects, characterizing coevolutionary processes. This thesis brought together three articles on the sorghum crop aiming at resistance to the main pests. In the first article, the objective was to evaluate the susceptibility of sorghum *bmr*-6, a mutant genotype with a lower concentration of lignin, to the pests *Diatraea saccharalis* and *Spodoptera frugiperda*. Experiments were carried out in laboratory and greenhouse, evaluating the development of these pests in sorghum biomass hybrids *bmr* 007, 008, TX635 and their respective conventional isogenic genotypes (without the *bmr* gene). The lignin content was higher in non-*bmr* hybrids. The lowest survival of *S. frugiperda* was verified in the hybrid BR008 both *bmr* and non-*bmr*. Injury scores by *S. frugiperda* in sorghum in greenhouse were high (>7) in all hybrids. For *D. saccharalis*, there was no significant difference for larval survival in the laboratory, but in the greenhouse the hybrid BR007 both *bmr* and non-*bmr* provided greater survival. The second article aimed to evaluate the influence of *D. saccharalis* infestation on grain production in commercial hybrids. For this purpose, grain sorghum hybrids were planted in three crops: March 2018 (2018/1) (CROP SEASON 1), September 2018 (2018/2) (CROP SEASON 2) and March 2019 (2019/1) (CROP SEASON 3). In the experiment, six treatments were used, being the three hybrids of grain sorghum and the same three hybrids with chemical treatment with the product Altacor® (150 ml ha⁻¹), under natural infestation, in four replications. The parameters evaluated were: length of galleries (cm), plant height (cm), length (cm) and weight (g) of panicles, infestation intensity and productivity. The infestation intensity of the three crops seasons was higher when not treated with insecticide and resulted in lower productivity. Regarding the difference between crops seasons, the planting of the 1st crop season showed lower infestation intensity. The hybrid DKB 590 had the lowest tolerance level, and with insecticide production increased 1.5 times in crop season 1 (149.77%). In crop season 2, hybrid BRS 373 showed the smallest increase in grain yield with control (29.79%), followed by AG1090 with 33.13%, and again DKB 590 (40.79%) with greater dependence on chemical control to increase productivity. Thw crop season 3 had the lowest yield among the three crops, and the hybrid AG1090 responded better to chemical control with a 105.90% increase in yield, followed by DKB 590 with 54.26% and BRS373 with 38.19%. The third article evaluated the susceptibility of 30 grain sorghum hybrids to *S. frugiperda*, *D. saccharalis* and *Diceraeus melacanthus* in a greenhouse. For fall armyworm, plant evaluation was performed 7 and 14 days after infestation (DAI). For *D. saccharalis* at 40 DAI and for the green-bellied stink bug at 12, 19 and 26 days after infestation. Through cluster analysis, it was possible to separate grain sorghum hybrids into groups according to the levels of resistance to each pest. The BRS373 hybrid stood out as moderately resistant to *S. frugiperda*; AG1090, 80G20, BRAVO, BRS373, AG1615 and IG220 were the most promising for *D. saccharalis*; and for *D. melacanthus*, the hybrids 50A40, A9735R, JADE, ENFORCER, BUSTER, 50A10 and IG244 were the most indicated. It is concluded that sorghum is susceptible to the three species studied here, the selection of hybrids is specific for each species, the lower lignin content in sorghum does not make the plant more susceptible to *D. saccharalis* and *S. frugiperda*, and grain sorghum it can be very sensitive to *D. saccharalis* attack, decreasing productivity by up to 1.5 times depending on the hybrid, planting time, lack of monitoring and adequate control.

Keywords: Plant resistance. *Spodoptera frugiperda*. *Diatraea saccharalis*. *Diceraeus melacanthus*. grain sorghum. energy sorghum.

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PRIMEIRA PARTE

1 1 INTRODUÇÃO

2
3 As interações inseto-planta envolvem mecanismos de sobrevivência, selecionando
4 genes de resistência da planta e quebra dessa resistência pelos insetos num processo de
5 coevolução. Essas interações resultam no desenvolvimento de mecanismos de defesa nas
6 plantas. Como por exemplo, as defesas químicas as quais fazem pressão de seleção sobre os
7 insetos. Estes, por sua vez, desenvolvem mecanismos de desintoxicação das defesas químicas,
8 proporcionando nova pressão de seleção sobre as plantas, que contra-atacam com a produção
9 de outros compostos químicos (EHRLICH; RAVEN, 1964). Dessa forma, estudar os padrões
10 comportamentais e biológicos de insetos-praga em suas plantas hospedeiras torna-se necessário
11 para o entendimento da preferência e desenvolvimento do inseto, bem como da resposta da
12 planta diante da injúria (KNOLHOFF; HECKEL, 2013), e assim. proporcionar conhecimentos
13 para aplicação da tática de resistência de plantas no Manejo Integrado de Pragas (MIP).

14 A resistência de plantas a insetos, em função de sua natureza poligênica na maioria dos
15 casos, pode se manifestar em diferentes níveis: alta resistência, moderada resistência,
16 suscetibilidade e alta suscetibilidade (BALDIN et al.,2019). A resistência de plantas, seja de
17 forma convencional ou transgênica, é uma das táticas preconizadas dentro do MIP, uma vez
18 que apresenta como uma das principais vantagens a associação compatível com as demais
19 táticas de manejo. Como é conhecido, as plantas possuem diferenças quanto à suscetibilidade a
20 pragas. Isto é, um fenótipo pode apresentar maior ou menor resistência (ou suscetibilidade) ao
21 ataque de insetos-praga, que pode ser expressa de forma constitutiva ou induzida
22 (GATEHOUSE, 2002; BASTOS et al., 2015). Em todas as culturas cultivadas em alta escala é
23 possível trabalhar a resistência de plantas a insetos.

24 O sorgo, *Sorghum bicolor* (L.) Moench, é o quinto cereal mais produzido no mundo,
25 usado como grãos, forragem e energia. Pelos diversos tipos de sorgo e sua ampla variabilidade
26 genética, estudos de avaliação de resistência ainda são insipientes. Além disso, a resistência de
27 plantas se adapta e integra de forma compatível às demais táticas de controle de pragas dentro
28 dos preceitos do MIP, possui interação com o controle biológico e químico, aumentando sua
29 eficiência, interferindo menos com as outras táticas de controle e com o ambiente.

30 O sorgo biomassa é uma matéria-prima lignocelulósica promissora por ser uma cultura
31 produtiva e adaptada a condições de estresse hídrico, o que é uma vantagem em um cenário que
32 demanda economia de água, além de ter um sistema de produção já bem conhecido e ser
33 adaptado a diferentes condições ambientais (SILVA et al. 2017). O sorgo biomassa *brown*
34 *midrib mutant (bmr)* é uma alternativa ao setor energético para uso em processos em que a

35 lignina representa um obstáculo, como por exemplo para a produção de etanol de segunda
36 geração. Para esta finalidade, a matéria-prima precisa ter baixo teor de lignina, como nesse
37 mutante de sorgo *bmr*, pois a celulose é um polímero de glicose que necessita ser quebrado para
38 este processo. No entanto, a lignina nas plantas pode ser um fator de resistência às pragas,
39 sobretudo lepidópteros (DOW et al. 2016).

40 O sorgo granífero pode substituir o milho em rações para alimentação animal, como
41 alimento base de pessoas em regiões da África e Ásia, além de ser promissor para alimentação
42 humana em função de ser rico em compostos bioativos, funcionais e não conter glúten
43 (WAQUIL, et al., 2003; FAO, 1995; MARTINO et al., 2014. O Brasil é atualmente o 9º maior
44 produtor mundial, sendo responsável por aproximadamente 10% da produção de sorgo
45 granífero do mundo. Na safra 2019 no país foram produzidas quase 2,5 milhões de toneladas
46 de grãos de sorgo, com destaque para o estado de Goiás que representa mais de 40% da
47 produção nacional, seguido por Minas Gerais (IBGE, 2020).

48 A ocorrência de insetos-praga representa um problema à cultura do sorgo. A broca-da-
49 cana *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) é uma das principais
50 pragas da cultura, cujo maior prejuízo para uma planta que pode alcançar cinco metros de altura
51 é tornar o colmo frágil e suscetível ao tombamento, além de dificultar o fluxo de seiva e
52 fotoassimilados na planta (MENDES et al. 2014; SILVA et al. 2017). A lagarta-do-cartucho
53 *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) também é uma das principais
54 pragas da cultura do sorgo, causando desfolha e reduzindo a área foliar para fotossíntese.
55 Devido ao cultivo de milho ou sorgo na segunda safra agrícola (“safrinha”) em sucessão à soja,
56 pragas que anteriormente eram consideradas secundárias têm aumentado a ocorrência em
57 lavouras nos sistemas de plantio direto, como é o caso do percevejo-barriga-verde *Diceræus*
58 *melacanthus* (Dallas, 1851) (Hemiptera: Pentatomidae). O percevejo barriga-verde tem o hábito
59 de se alimentar e reproduzir sob a palhada das culturas antecedentes, e causa danos
60 significativos às plantas de milho e sorgo no início do desenvolvimento vegetativo, reduzindo
61 o vigor e além de poder causar a morte das plantas (CONAB, 2020; GASSEN, 1996; CORRÊA-
62 FERRERA; SOSA-GOMÉZ, 2017).

63 Neste sentido, este trabalho foi dividido em três artigos. O primeiro objetivou avaliar a
64 suscetibilidade do sorgo *bmr-6* à lagartas de *D. saccharalis* e *S. frugiperda* e se o menor teor
65 de lignina nas plantas favorece ou não a alimentação e desempenho das pragas. O conhecimento
66 dessas informações é de grande importância, uma vez que a redução de lignina na matéria-
67 prima bioenergética poderia causar maior suscetibilidade a insetos-praga pelo fato de esse
68 composto fenólico representar um mecanismo de defesa de plantas a insetos herbívoros

69 (DOWD et al. 2016; VENDRAMIM and GUZZO 2019). No segundo artigo, o objetivo foi
70 conhecer o potencial de dano da praga na cultura do sorgo em três dos materiais comerciais
71 mais utilizados, bem como se variam quanto aos níveis de resistência para auxiliar no
72 desenvolvimento de estratégias de MIP na cultura do sorgo. E o terceiro artigo, o objetivo foi
73 avaliada a suscetibilidade de 30 híbridos de sorgo granífero a três importantes pragas da cultura,
74 *S. frugiperda*, *D. saccharalis* e *D. melacanthus*.

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94 2 REFERENCIAL TEÓRICO

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96 2.1 A CULTURA DO SORGO E SUAS PRAGAS

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98 O sorgo é considerado uma planta rústica que tem boa adaptação a diferentes condições
99 ambientais, podendo produzir bem em condições que seriam desfavoráveis a maioria dos outros
100 cereais (SILVA et al., 2017). Existem diferentes tipos de sorgo: o granífero, destinado à
101 produção de grãos; o forrageiro, para produção de silagem; o sacarino, para produção de etanol;
102 o sorgo vassoura, utilizado para fabricação de vassouras; e o biomassa, usado para geração de
103 energia em usinas termelétricas (VILELA et al., 2017). Além disso, as plantas e lavouras com
104 as diferentes aptidões supracitadas possuem porte, ciclo de cultivo e tratos culturais
105 diferenciados. Nesse sentido, as infestações de insetos-praga podem ter importâncias distintas
106 dependendo do grupo de sorgo. Também, os genótipos de sorgo dentro de cada grupo podem
107 apresentar diferenças em características bioquímicas, como no teor de açúcares solúveis, lignina
108 e minerais (CHUPIN et al., 2017), e estas características podem afetar a resistência a insetos-
109 praga.

110 Na fase inicial de desenvolvimento do sorgo, a cultura pode ser atacada por diversas
111 espécies de insetos praga. Entre os mais importantes se destacam os corós (*Phyllophaga* spp.,
112 *Stenocrates* spp., *Cyclocephala* spp., *Diloboderus* spp.) (Coleoptera: Scarabaeidae), cupins
113 (*Heterotermes* spp., *Syntermes* spp., *Proconitermes* spp.) (Blatodea: Termitidae), larvas-
114 alfinetes *Diabrotica speciosa* (Germar, 1824) e *Diabrotica viridula* (Fabricius, 1801)
115 (Coleoptera: Chrysomelidae), percevejos-castanhos *Scaptocoris castanea* (Petry, 1830) e
116 *Atarsocoris brachiariae* (Becker, 1996) (Hemiptera: Cydnidae), percevejo-preto *Cyrtomenus*
117 *mirabilis* (Hemiptera: Cydnidae), lagarta-elasma *Elasmopalpus lignosellus* (Zeller, 1848)
118 (Lepidoptera: Pyralidae), larva-aramé *Conoderus escalaris* (Germar, 1824) (Coleoptera:
119 Elateridae), larva-angorá *Astylus variegatus* (Germar, 1824) (Coleoptera: Dasytidae) e os
120 percevejos-barriga-verde *Dicereaus furcatus* (Fabricius, 1775) e *Dicereaus melacanthus*
121 (Dallas, 1851) (Hemiptera: Pentatomidae) (MENDES et al., 2014).

122 Na fase vegetativa do sorgo podem-se encontrar os pulgões *Rhopalosiphum maidis*
123 (Fitch, 1856) e *Schizaphis graminum* (Rondani, 1852) (Hemiptera: Aphididae), lagarta-do-
124 cartucho *Spodoptera frugiperda* (J. E. Smith, 1797), curuquerê-dos-capinzais *Mocis latipes*
125 (Guennée, 1852) (Lepidoptera: Noctuidae), e a broca-do-colmo *Diatraea saccharalis*
126 (Lepidoptera: Crambidae).

127 Por fim, na fase reprodutiva merecem destaque a mosca-do-sorgo *Stenodiplosis*
 128 (=*Contarinia sorghicola*) (Coquillett) (Diptera: Cecidomyiidae), os percevejos-da-panícula
 129 *Leptoglossus zonatus* (Dallas, 1852 (Hemiptera: Coreidae), *Nezara viridula* (Linnaeus, 1758),
 130 *Thyanta perditor* (Fabr., 1794) (Hemiptera: Pentatomidae), *Sthenaridea carmelitana*
 131 (Carvalho, 1948) (Hemiptera: Miridae), e a lagarta *Helicoverpa zea* (Boddie, 1850)
 132 (Lepidoptera: Noctuidae) (WAQUIL; VIANA; CRUZ, 2003).

133 Na região Sudeste e Centro Oeste do Brasil, geralmente a cultura do sorgo é implantada
 134 no período de segunda safra, logo após a colheita de soja precoce. Isto faz com que pragas
 135 remanescentes nos restos culturais possam migrar para as culturas do sorgo e do milho, como
 136 tem recentemente ocorrido com o percevejo-barriga-verde *D. melacanthus*. Este percevejo
 137 causa danos significativos às plantas de milho e sorgo no início do desenvolvimento vegetativo,
 138 reduzindo o vigor, promovendo o perfilhamento precoce e pode até matar a planta (WAQUIL;
 139 OLIVEIRA, 2009; CORREA-FERRERA et al, 2017).

140 Na safra 2018/2019, foi observada nos plantios de sorgo, principalmente em sorgo
 141 granífero, alta infestação atípica do pulgão da cana-de-açúcar *Melanaphis sacchari* (Zethner,
 142 1897) (Hemiptera: Aphididae). Este pulgão é considerado como uma forte ameaça à cultura do
 143 sorgo não só no Brasil, como nos Estados Unidos desde 2013 (PAUDYAL et al., 2019). Entre
 144 todas as pragas do sorgo, a broca-do-colmo *D. saccharalis* é considerada a mais importante
 145 para o sorgo sacarino por causar injúrias na parte econômica da planta, que é o colmo.
 146 Juntamente com *S. frugiperda*, *D. melacanthus*, *S. graminum*, e mais recentemente *M. sacchari*
 147 são consideradas as pragas-chave da cultura do sorgo, ou seja, as que demandam mais atenção
 148 no monitoramento e controle.

149 O ataque de pragas pode ser um importante fator na perda de produtividade do sorgo,
 150 reduzindo a expressão do seu potencial. Dessa forma, torna-se muito importante o uso de
 151 diferentes táticas de controle no manejo integrado das pragas dessa cultura (MENDES et al.,
 152 2014). Diante disso, selecionar genótipos resistentes ou tolerantes às principais pragas do sorgo
 153 é de suma importância para programas de melhoramento genético e de MIP.

154

155 2.2 *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae)

156

157 A lagarta-do-cartucho *S. frugiperda* possui distribuição geográfica generalizada,
 158 causando danos durante, praticamente, em todas as fases de desenvolvimento do milho
 159 (DEQUECH et al., 2013). Em geral, o sorgo é mais resistente que o milho à lagarta-do-cartucho.
 160 Entretanto, há cultivares de sorgo que são tão suscetíveis a essa espécie quanto o milho.

161 Condições tropicais de cultivo aliadas as características dessa espécie como polifagia e alta
162 capacidade de reprodução, dispersão e migração dos adultos (NAGOSHI; MEAGHER, 2008),
163 favorecem a ocorrência de gerações sobrepostas ao longo do ano, levando a graves infestações
164 em safras agrícolas (BERNARDI et al., 2015).

165 O adulto de *S. frugiperda* é uma mariposa com aproximadamente 3,5 cm de
166 comprimento, coloração pardo-escura nas asas anteriores e branco-acinzentadas nas
167 posteriores, com longevidade de aproximadamente 15 dias (SANTOS et al., 2004). As posturas
168 são feitas em massa, com média de 150 ovos. O período para eclosão das larvas é de
169 aproximadamente três dias.

170 As larvas recém-eclodidas alimentam-se do próprio córion dos ovos e, posteriormente
171 das folhas da planta, provocando o sintoma conhecido como “folhas raspadas”, o que é um
172 sintoma da presença da lagarta na cultura. As lagartas passam por cinco ou seis estádios larvais,
173 atingindo 40 a 50 mm de comprimento e 2,7 a 2,78 mm de largura da cápsula cefálica. À medida
174 que as lagartas crescem, começam a fazer orifícios nas folhas, podendo causar severas injúrias
175 às plantas (CRUZ; ALVARENGA; FIGUEIREDO, 1995). A duração da fase larval é de 12 a
176 30 dias. Após este período as lagartas se direcionam para o solo onde passarão à fase de pupa,
177 que dura entre 10 a 12 dias. O ciclo biológico desse inseto em plantas de milho completa-se
178 em 25 dias à temperatura de 25°C, aumentando o número de dias quando as temperaturas estão
179 mais baixas (BUSATO; GRUTZMACHER; GARCIA, 2005a; BUSATO; GRUTZMACHER;
180 GARCIA, 2005b).

181 Os primeiros trabalhos que tentaram identificar fontes de resistência do sorgo à lagarta-
182 do-cartucho foram realizados por McMillian & Starks (1967) nos EUA e por Lucena (1978) no
183 Brasil. Mesmo considerando o pequeno número de genótipos avaliados foi possível identificar
184 tipos contrastantes como AF-28 (menor índice de infestação) e EA 261 (menor consumo de
185 biomassa). Entre 20 variedades de sorgo sacarino, foi observada uma redução no consumo das
186 lagartas de 44% da área foliar da variedade Honey quando comparada à variedade Dale
187 (WAQUIL; SANTOS, 1990). A avaliação de 18 acessos de sorgo selecionados, previamente,
188 para resistência à lagarta-do-cartucho, de 326 acessos do Banco Ativo de Germoplasma da
189 Embrapa Milho e Sorgo, Santos et al. (1992) identificaram como resistentes: AF 28; MN 1533;
190 IS 5831, que causaram 67% de mortalidade de larvas, e Tx 7078 e BTx 611 wx, que
191 apresentaram menor área foliar consumida (SANTOS et al., 2005).

192 Como a distribuição da infestação natural no campo é muito irregular a seleção de
193 híbridos sob infestação natural fica prejudicada. Desta forma, como a lagarta-do-cartucho pode
194 ser criada em laboratório, os trabalhos mais recentes têm sido conduzidos com infestação

195 artificial, que podem ser conduzidos em laboratório, casa-de-vegetação ou no campo (neste
196 caso já ocorre a infestação natural). Para avaliar a resistência à desfolha por *S. frugiperda*
197 existem escalas de notas de injúria, de modo que de acordo com a intensidade da injúria maior
198 será a nota atribuída. Entre as escalas de injúria no milho destacam-se a de 0-5 desenvolvida
199 por Carvalho (1970) e a de 1-9 desenvolvida por Davis (1992). Gonçalves et al. (2011)
200 avaliaram através de escala de notas (0-5) genótipos de sorgo sacarino em casa-de-vegetação
201 aos 14 dias após a infestação das lagartas. Segundo os autores, o índice de adaptação das
202 lagartas aos genótipos de sorgo sacarino seguiu a seguinte ordem decrescente (do mais
203 adaptável aos menos adequados à lagarta-do-cartucho): BR505, CMSX5634, BR501,
204 CMSX5642 e CMSX5630.

205 Na avaliação da adubação nitrogenada em sorgo, López et al. (2000) não verificaram
206 correlação significativa entre parâmetros de desenvolvimento da lagarta-do-cartucho e a
207 concentração total de nitrogênio nas plantas. Ao avaliar três cultivares comerciais de sorgo
208 quanto à infestação de *S. frugiperda*. Cortez; Waquil (1997) encontraram grande variação nos
209 parâmetros avaliados, sugerindo que haja constante avaliação de genótipos com potencial para
210 se utilizar a resistência do sorgo no MIP.

211

212 2.3 *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae)

213

214 A broca-do-colmo ou broca-da-cana-de-açúcar *D. saccharalis* é uma praga primária
215 para a cana-de-açúcar, mas por ser polífaga se alimenta de outras culturas e tem causado perdas
216 significativa no milho, trigo, sorgo e milheto (MENDES et al., 2014). Pelo comportamento
217 alimentar formando galerias, a praga se encontra protegida, dificultando o controle químico.
218 Dessa forma, a utilização de genótipos resistentes e tolerantes são as táticas de controle mais
219 promissoras para o manejo de *D. saccharalis* na cultura do sorgo (MENDES et al., 2014;
220 SANTOS; MIHSFELDT, 2014). Na cultura do sorgo ela pode danificar tanto o colmo como o
221 pedúnculo da panícula. Quando a injúria é no colmo, causa quebramento e redução na produção
222 e quando é no pedúnculo causas morte da panícula provocando o sintoma de panícula-branca,
223 com 100% de perda da planta atacada no caso do sorgo grânifero.

224 As galerias provocadas pelas lagartas de *D. saccharalis* deixam as plantas mais
225 vulneráveis à queda pela ação do vento, o que é um prejuízo indireto muito importante: o colmo
226 fica enfraquecido, podendo quebrar ou tombar. O ataque da broca-do-colmo causa podridão por
227 favorecer a entrada de microrganismos, causando o acúmulo de micotoxinas, morte da gema

228 apical, redução do fluxo de seiva, encurtamento dos entrenós dos colmos e acamamento,
229 debilitando a planta de forma geral (MENDES et al., 2014; SILVA et al., 2014).

230 O adulto é uma mariposa de coloração amarelo-palha, de 20 mm de envergadura. Os
231 ovos têm aspecto de escamas; inicialmente possuem coloração amarelo-claro, alaranjado e vão
232 escurecendo quando perto da eclosão da lagarta. Os ovos são colocados nas folhas e a eclosão
233 ocorre entre quatro e nove dias. Inicialmente, as lagartas se alimentam das folhas, passam para
234 a bainha e depois se direcionam para o colmo, fazendo galerias ascendentes e originando um
235 orifício por onde a mariposa emerge, que pode variar de nove a 14 dias (MENDES et al., 2014).

236 A maioria dos trabalhos com avaliação de genótipos de sorgo para a resistência a *D.*
237 *saccharalis* e outros lepidópteros no país foram realizados há mais de 15 anos. Dentre esses
238 trabalhos, Bortoli et al. (2005) ao avaliarem o efeito da adubação em uma cultivar de sorgo no
239 desenvolvimento de *D. saccahralis* observaram que as menores porcentagens de injúria foram
240 verificadas com as menores doses de nitrogênio e maiores doses de potássio.

241 Normalmente, as avaliações são baseadas na taxa de plantas com panículas mortas, nos
242 índices de infestação (% plantas infestadas) e de intensidade de infestação (% de internódios
243 danificados). Para se avaliar esses índices, deve-se abrir o colmo longitudinalmente e examinar.
244 Pode-se utilizar também uma escala visual de notas (variando de 0 a 9 com base na área foliar
245 consumida para se estimar as injúrias causadas pelas lagartas nas folhas. Muitas fontes
246 demonstraram resistência cruzada a diferentes espécies de brocas, como os IS 1055 (BP53), IS
247 1044, IS 2123, IS 2195, IS 2205, IS 5469 e IS 18551.

248 A variedade Brandes, AF 28 e SC 3541 foram consideradas as mais resistentes à broca-
249 do-colmo (Waquil et al., 1980; Boiça Jr.; Lara, 1983; Lara et al., 1997). Entre as cultivares
250 comerciais, os resultados mostraram que o BR 304 e o CMSXS9701 apresentaram os menores
251 índices de infestação enquanto que Z 732 e Esmeralda destacaram-se com os menores índices
252 de intensidade de infestação (WAQUIL et al., 2001). Os autores encontraram alta variabilidade
253 genética entre os materiais comerciais, e perdas significativas na produtividade podem ocorrer
254 em todos os híbridos avaliados. Por outro lado, a variabilidade genética observada entre as
255 linhagens de sorgo indica grande potencial para a seleção de cultivares resistentes nos
256 programas de melhoramento (WAQUIL et al., 2001).

257 Lara et al. (1979) encontraram resistência em dois genótipos de sorgo entre 89 avaliados
258 para *D. saccharalis*, e após quase duas décadas também foram encontrados dois genótipos com
259 resistência induzida indireta (denominada pelo autor de resistência extrínseca) a *D. saccharalis*,
260 considerando o maior parasitoidismo por *Cotesia flavipes* (Cameron, 1891) (Hymenoptera:
261 Braconidae) (LARA et al., 1997). Genótipos de sorgo, 10 híbridos de sorgo sacarino e 16 de

262 sorgo biomassa foram avaliados em relação à resistência a *D. saccharalis*, com destaque para
263 o genótipo CMSXS647 pelas características altura de plantas, nível de infestação, tamanho de
264 galerias e teor de sólidos solúveis entre os sacarinos, enquanto que entre os genótipos de sorgo
265 biomassa, destacaram CMSXS7030, CMSXS7012 e CMSXS7028, os quais apresentaram
266 características desejáveis para nível de infestação, altura de plantas e número de compostos
267 lignocelulósicos (ARAÚJO et al., 2019). Mais trabalhos de levantamento de genótipos e
268 híbridos resistentes ou tolerantes a esta praga são necessários.

269

270 **2.4 *Diceraeus melacanthus* (Dallas, 1851) (Hemiptera: Pentatomidae)**

271

272 Na região Sudeste e Centro Oeste do Brasil, geralmente a cultura do sorgo é implantada
273 no período de segunda safra, logo após a colheita de soja precoce. Nesse contexto, pragas que
274 anteriormente eram consideradas secundárias vêm recebendo grande atenção pelo elevado
275 crescimento populacional nos sistemas de cultivo direto. Isto faz com que pragas remanescentes
276 nos restos desta cultura possam migrar para as culturas do sorgo e do milho, como é o que tem
277 acontecido nas últimas safras com o percevejo-barriga-verde *D. melacanthus*. Este percevejo
278 causa injúrias significativas às plantas de milho e sorgo no início do desenvolvimento
279 vegetativo, reduzindo o vigor, promovendo o perfilhamento precoce e pode até matar a planta
280 (WAQUIL; OLIVEIRA, 2009; CORREA-FERRERA et al., 2017).

281 O percevejo barriga-verde tem o hábito de se alimentar e reproduzir sob a palhada das
282 culturas antecedentes, e causa injúrias significativas às plantas de milho e sorgo no início do
283 desenvolvimento vegetativo, reduzindo o vigor e além de poder causar a morte das plantas
284 (GASSEN, 1996; CORRÊA-FERRERA; SOSA-GOMÉZ, 2017). Ao se alimentar,
285 normalmente se posiciona na base do colmo da planta hospedeira com a cabeça voltada para
286 baixo, e introduz os estiletes para sugar a seiva do floema, cuja succão é facilitada pela injeção
287 de enzimas salivares que são tóxicas às plantas, o que prejudica seu desenvolvimento
288 (BIANCO, 2004; GRIGOLLI et al., 2016).

289 A planta é mais suscetível às injúrias causadas por *D. melacanthus* nos primeiros dias
290 após a emergência das plantas (BIANCO, 2004), provavelmente por ser a fase mais sensível
291 que se define o potencial produtivo da cultura. Nesse sentido a intensidade do dano pode variar
292 com o número de percevejos na lavoura também com a escolha do híbrido e do manejo adotado.
293 Estudos com *D. melacanthus* já foram propostos para cultura do milho, mas na cultura do sorgo
294 ainda são insipientes (BIANCO, 2004; ROZA-GOMÉZ et al., 2011; DUARTE et al., 2015;
295 CRUZ et al., 2016; FERNANDE et al., 2020; BUENO et al., 2021).

296 **2.5 Sorgo biomassa (*bmr*)**

297
298 O sorgo biomassa apresenta rápido crescimento e alta produção, podendo atingir 150
299 toneladas ha⁻¹. As plantas podem chegar até 6 m de altura em 180 dias (PARRELLA, 2013).
300 Além disso, possui cultivo totalmente mecanizado e poder calorífero parecido com o da cana-
301 de-açúcar, eucalipto e capim-elefante (MAY, 2013). O sorgo biomassa pode ser utilizado em
302 usinas termelétricas e em indústrias que utilizam caldeiras para geração de energia para
303 consumo próprio, visando ao fornecimento de matéria-prima para cogeração de energia e/ou
304 produção de etanol de segunda geração. Por ser uma cultura relativamente nova para essa
305 finalidade, pesquisas com os mais variados propósitos devem ser realizadas, e dentre elas, cabe
306 à investigação das defesas das plantas de sorgo biomassa ao ataque de insetos-praga (MAY,
307 2013; PARRELLA, 2013).

308 No âmbito de geração de energia, biomassa é qualquer produto ou coproduto de um
309 recurso renovável procedente de matéria orgânica basicamente formada por hidratos de carbono
310 e que pode ser utilizada na produção de energia (CCEE, 2021). A parede das células vegetais é
311 composta por três grandes polímeros: a celulose, que constitui a maior proporção, a
312 hemicelulose, que é a segunda mais abundante, e a lignina. A celulose é uma cadeia de
313 moléculas de glicose bem organizada, enquanto a hemicelulose não apresenta uma
314 padronização em sua estrutura; existem outros açúcares em sua constituição além da glicose,
315 como manose, xilose, arabinose e galactose, além de alguns ácidos (RUBIN, 2008). A lignina
316 é formada por três componentes fenólicos principais: álcool p-coumaryl, álcool coniferílico e
317 álcool sinapílico; são polímeros aromáticos que variam em suas ramificações e se condensam
318 em diferentes estruturas. Os três grandes polímeros presentes na parede celular das plantas são
319 organizados em microfibrilas que dão estabilidade estrutural à parede celular (RUBIN, 2008).

320 O colmo das plantas também é constituído de celulose, hemicelulose e lignina, além de
321 cinzas que é a parte mineral, que dá sustentação ao colmo (SANTOS et al., 2011). Como o
322 sorgo biomassa possui alto teor de lignina, pode ser utilizado para cogeração de energia, pois a
323 lignina aumenta o poder calorífero necessário para a queima. Dentro da categoria biomassa,
324 existe o sorgo *bmr* (*brown midrib*), que é um genótipo mutante caracterizado pela presença de
325 nervura das folhas com coloração amarronzada. Essa mutação foi primeiramente observada
326 naturalmente em plantas de milho (JORGENSEN, 1931), e desde então foi induzida no sorgo
327 através de tratamento químico com o uso de metano sulfonato de etila. A mutação faz com que
328 o teor de lignina na planta de sorgo reduza substancialmente (XIN et al., 2008). Dessa forma,
329 sua utilização torna-se ideal para a produção de etanol de segunda geração, em que a lignina é

330 um impedimento para a sacarificação e fermentação da biomassa em etanol (DIEN et al., 2009).
331 Além disso, pode proporcionar melhor digestibilidade ao gado (CHERNEY et al., 1991).

332 Para produção de etanol de segunda geração no Brasil, a matéria-prima largamente
333 utilizada é o bagaço de cana-de-açúcar (CCEE, 2021). No entanto, apesar da sacarídea estar
334 muito bem estabelecida, são necessárias mais alternativas para compor a matriz energética
335 nacional. O sorgo biomassa é uma matéria-prima lignocelulósica promissora por ser uma
336 cultura rústica, ter um sistema de produção já bem conhecido, apresentar adaptação a diferentes
337 condições ambientais e tolerar deficiência hídrica, o que é uma vantagem em um cenário que
338 demanda economia de água (SILVA et al., 2017). Com a escassez de chuvas, há redução na
339 oferta de energia proveniente das hidrelétricas, demandando geração térmica por biomassa,
340 cenário ideal para a participação do sorgo biomassa (CCEE, 2021). O sorgo biomassa *bmr* tem
341 importância também para alimentação animal, o valor nutritivo é maior, o menor teor de lignina
342 facilita a digestibilidade (EBLING & KUNG Jr. 2004). Com a redução da lignina e aumento da
343 digestibilidade da FDN é um parâmetro importante para qualidade da forragem, com a melhor
344 digestão da parte fibrosa, reduz o efeito do enchimento do rúmen e aumenta o consumo de
345 matéria seca (OBA & ALLEN, 1999).

346 Apesar da rusticidade do sorgo, o ataque de insetos-praga representa um problema à
347 cultura. A broca-do-colmo *D. saccharalis* é uma das principais pragas da cultura do sorgo, cujo
348 maior prejuízo é deixar o colmo frágil e suscetível ao tombamento, reduzindo o fluxo de seiva
349 na planta, entre outros problemas (MENDES et al., 2014; SILVA et al., 2014). Portanto, como
350 o sorgo biomassa é muito volumoso, com porte de 5-6 m de altura, se houver infestação da
351 broca-do-colmo a probabilidade de tombamento será ainda maior. Além disso, pelo fato de o
352 sorgo *bmr* ter menor teor de lignina, é naturalmente mais suscetível ao tombamento devido à
353 maior fragilidade estrutural. (PARRELLA, 2013). Portanto, torna-se necessário verificar se o
354 menor teor de lignina nas plantas favorece ou não a oviposição, alimentação e desenvolvimento
355 das pragas, uma vez que a redução de lignina na matéria-prima bioenergética poderia causar
356 suscetibilidade a insetos-pragas pelo fato de esse composto fenólico representar um mecanismo
357 de defesa de plantas a herbívoros (DOWD; FUNNELL-HARRIS; SATTLER, 2016;
358 VENDRAMIM; GUZZO, 2019).

359

360

361

362

363

364 **2.6 Sorgo granífero**

365

366 O sorgo granífero é uma importante cultura de cereal usado como grão e pode substituir
367 o milho em rações para alimentação animal, como para alimentação humana em regiões da
368 África e Ásia, além de ser promissor para alimentação humana em função de ser rico em
369 compostos bioativos, funcionais e não conter glúten (WAQUIL, et al., 2003; FAO, 1995;
370 MARTINO et al., 2014). Diante disso, tem crescido a demanda por produtos à base de sorgo
371 como massas para pães, macarrão, barras de cereais, “snacks”, bolos, biscoitos e cerveja; todos
372 estes produtos apresentam alto valor agregado pelos benefícios à saúde humana como
373 alternativa aos cereais convencionais (MARTINO et al., 2014; ABDELGHAFOR et al., 2011;
374 BURDETE et al., 2010; CARDOSO et al., 2014). O sorgo granífero contribui para uma oferta
375 de menor custo de grãos em épocas de menor pluviosidade e possui baixa suscetibilidade a
376 micotoxinas que é comum no milho e faz com que seja um cereal seguro para composição de
377 rações de modo geral (MENEZES et al., 2015).

378 Na safra 2020 no País foram produzidas quase 2,5 milhões de toneladas de grãos de
379 sorgo, com destaque para o estado de Goiás que representa mais de 40% da produção nacional,
380 seguido por Minas Gerais (IBGE, 2020). O sorgo geralmente é plantado na segunda safra em
381 sucessão a cultura da soja. O plantio varia entre fevereiro e abril dependendo da região do país.
382 O Centro-Oeste e Sudeste são os principais responsáveis pela produção de sorgo no Brasil. O
383 levantamento de produção estimada é de produzir 1,29 milhão de toneladas para safra
384 2020/2021. O Estado de Goiás é o maior produtor de sorgo do país representando 49,7% da
385 produção nacional. A expectativa é de aumento de 17,5% na produção, em comparação com a
386 safra (CONAB, 2021). No ranking mundial de produção de sorgo, os Estados Unidos é o
387 principal produtor, seguido de países da África, México, Argentina, Brasil, Índia, Pasquistão,
388 China, Austrália e União Européia (USDA, 2021).

389

390 **2.7 Resistência de plantas a insetos**

391

392 A resistência de plantas é a soma relativa de qualidades hereditárias possuídas pela
393 planta, as quais influenciam o resultado do grau de dano causado por um inseto (PAINTER,
394 1951). Assim, planta resistente pode ser definida como aquela que, devido a sua constituição
395 genotípica, é menos danificada que outras, consideradas suscetíveis, em igualdade de condições
396 (ROSSETTO, 1973).

397 Alguns princípios básicos da resistência de plantas a insetos devem ser observados. A
398 resistência é relativa, o que implica na comparação de duas ou mais plantas. Dessa forma,
399 quando se diz que uma planta é resistente a um determinado inseto significa que ela manifesta
400 esta resistência em relação a outras plantas. A resistência é hereditária, isto é, trata-se de um
401 caráter genético, de modo que as progêneres de uma planta resistente devem expressar a
402 resistência da mesma forma quando testadas nas condições em que a resistência se revelou. A
403 resistência é específica. Ou seja, pode ser resistente a uma espécie de inseto e suscetível a uma
404 outra espécie. Também apresenta plasticidade fenotípica de acordo com a interação do genótipo
405 x ambiente, podendo alterar o fenótipo dependendo das condições ambientais. Isto pode
406 implicar, por exemplo, no nível expresso de resistência. Além disso, é necessário que haja
407 repetibilidade das respostas. Ou seja, todas as vezes que se testar a variedade resistente em
408 comparação com as outras variedades testadas, aquela característica deverá se manifestar
409 (LARA, 1991; BALDIN et al., 2019).

410

411 **2.7.1 Classificação da resistência de plantas a inseto**

412 Quanto às formas de expressão das características de resistência, podem ser
413 constitutivas e induzidas. A resistência constitutiva é de modo geral constantemente expressa
414 na planta, e assim os genes que participam da codificação de substâncias químicas,
415 características físicas e morfológicas são expressos em geral durante todos os estádios de
416 desenvolvimento da planta (SCHOONHOVEN et al., 2005). Por outro lado, a resistência
417 induzida ocorre após o reconhecimento de algum estímulo ou fator de estresse, com a ação de
418 elicidores como o ácido jasmônico e salicílico após a ocorrência de herbivoria ou oviposição,
419 infecção por patógenos ou outro tipo de estresse biótico ou abiótico (ZARATE et al., 2007).
420 Através do reconhecimento inicial destes elicidores por receptores de reconhecimento de
421 padrões moleculares, as plantas apresentarão uma resposta induzida de defesa, seja aumentando
422 quantitativamente a produção de tricomas, enzimas antioxidantes e de defesa, compostos
423 químicos tóxicos ou anti-nutricionais para o inseto, quanto expressão qualitativa dessas e outras
424 características (VENDRAMIM et al., 2019).

425 Além do modo de expressão dos genes e características de resistência, esta pode ser
426 classificada como direta, como por exemplo, espinhos, tricomas, formato, espessura da parede
427 celular, velocidade de crescimento celular, dureza dos tecidos atacados, incrustação de minerais
428 como silício na cutícula, presença de cera na superfície da folha, aumento da síntese de algum

429 composto secundário ou enzimas oxidantes e de defesa que têm efeitos diretamente no inseto
430 fitófago (HOFFMAN-CAMPOS; GRAÇA, 2019; VENDRAMIM et al., 2019).

431 Quando a planta é induzida, como por exemplo, pela herbivoria de insetos, desencadeia
432 mudanças na preferência, desempenho ou reprodução do inseto diante da planta hospedeira que
433 vai fazer com que se alimente menos ou tenha uma redução de peso, alteração no
434 desenvolvimento, e por fim, taxa de sobrevivência e oviposição, resultando crescimento
435 populacional (SCHOLZ et al., 2015). A resistência indireta compreende a produção de
436 compostos orgânicos voláteis que a planta libera e que atraem inimigos naturais dos herbívoros
437 que estão se alimentando daquela planta (SCHOONHOVEN et al., 2005). As plantas liberam
438 voláteis para comunicação entre elas e os herbívoros, orientando o comportamento de busca de
439 inimigos naturais como predadores e parasitoides, atraindo ou repelindo para a aceitação ou não
440 do hospedeiro, podendo afetá-los de diferentes formas, tanto na parte aérea quanto subterrânea
441 (STOUT, 2013). Existem também estruturas morfológicas produzidas de forma induzida nas
442 plantas como os nectários extraflorais presentes em folhas e caules que podem ser utilizados
443 como fonte de alimento para predadores e parasitoides, e as domácia que são estruturas
444 presentes em algumas plantas como cavidades ou tufos de pelos que servem como abrigo e
445 alimento para os inimigos naturais (SILVA; PANIZZI, 2019).

446 Do ponto de vista genético, a resistência pode ser: monogênica (um lócus), oligogênica
447 (poucos loci) ou poligênica (muitos loci). A interação pode ser intra-alélica – a expressão da
448 resistência pode ser recessiva; dominante ou com dominância incompleta ou inter-alélica – a
449 expressão da resistência pode depender de dois ou mais genes com ação complementar (sendo
450 um único insuficiente); pode ser aditiva (dois genes não-alélicos afetam a mesma característica)
451 e epistática (um gene inibe a ação de outro) (HSIEH & PI, 1982).

452 Em relação à resposta entre as populações de plantas e a espécie da praga, existe a
453 resistência horizontal que é quando a planta resistente expressa o mesmo nível de resistência,
454 contra todos os biótipos do inseto-praga (raças semelhantes morfologicamente, mas com
455 respostas diferenciadas em função da variação na população da planta) e vertical, quando a
456 resistência é específica para cada biótipo do inseto-praga (KOGAN 1975; LARA 1991;
457 BALDIN et al. 2019). Os fatores ambientais também podem afetar a expressão da resistência
458 como a temperatura, luminosidade, umidade relativa, estádio de desenvolvimento da planta ou
459 de seus tecidos, estado nutricional da planta e aplicação de defensivos e reguladores de
460 crescimento (LARA et al., 1991).

461

462 **2.7.2 Antixenose**

463 Antixenose é o tipo de resistência responsável pela não preferência do inseto para
464 alimentar, ovipositar ou se abrigar em um determinado fenótipo de planta (KOGAN;
465 ORTMAN, 1978). Os insetos possuem diversificação na preferência porque eles utilizam
466 estímulos fornecidos pelas plantas, os quais são responsáveis por desencadear respostas em
467 favor ou contrária à planta hospedeira. Os semioquímicos são estímulos químicos que regulam
468 ou mediam a seleção hospedeira por insetos e podem ser subdivididos em dois grandes grupos:
469 os aleloquímicos que são interespecíficos (comunicação entre indivíduos de espécies
470 diferentes) e os feromônios que são intraespecíficos (comunicação entre indivíduos de mesma
471 espécie).

472 Em termos da inter-relação inseto-planta, a importância deve ser atribuída ao grupo dos
473 aleloquímicos, pois são substâncias que traduzem um significado comportamental e biológico
474 a organismos pertencentes a espécies diferentes (BALDIN et al., 2019). Os aleloquímicos
475 subdividem-se em cairomônios, alomônios, sinomônios e apneumônios. Cairomônios são as
476 substâncias que, quando entram em contato com o indivíduo de outras espécies, como insetos,
477 causam uma reação comportamental ou fisiológica favorável ao receptor e não ao emissor. Por
478 outro lado, alomônios proporcionam o inverso da resposta, ou seja, é desfavorável ao receptor,
479 mas não ao emissor. Sinomônios são substâncias que em contato com o indivíduo de outra
480 espécie despertam uma reação comportamental ou fisiológica favorável a ambos, tanto ao
481 emissor quanto receptor. São exemplos relacionados à resistência indireta, pois favorecem os
482 inimigos naturais, atraindo-os, e também às plantas devido ao controle de pragas realizados
483 pelos inimigos naturais atraídos à planta. Por fim, apneumônios são compostos químicos que
484 são emitidos por matéria morta e que resultam em uma reação comportamental ou fisiológica
485 favorável ao receptor em um organismo que esteja dentro ou sobre o material emissor (BALDIN
486 et al., 2019; SILVA; PANIZZI, 2019).

487 Com relação às respostas desencadeadas pelos cairomônios das plantas aos insetos,
488 podem ser atraentes, atuando de forma que induz o inseto a se movimentar em direção à planta;
489 arrestante, quando induz o inseto a se agrigar à planta ou diminuir a locomoção para que eles
490 avaliem melhor a planta a curta distância; excitante ou incitante, quando leva o inseto a provar
491 a planta, ou seja, induz a picada ou “mordida” inicial, à penetração do ovipositor; e estimulante
492 de alimentação ou oviposição, quando induz o inseto a continuar se alimentando ou
493 ovipositando em uma planta adequada (BALDIN et al., 2019).

494 Os alomônios presentes nas plantas provocam reações inversas às provocadas pelos
495 cairomônios, isto é, são desfavoráveis aos insetos e favoráveis às plantas. Eles podem ser
496 classificados como repelentes, quando induzem o inseto a movimentar-se em direção oposta à
497 planta; estimulante de locomoção, quando induz o inseto a se afastar mais rapidamente da
498 planta; supressante ou supressor, quando inibe ou evita o início da alimentação (picada ou
499 “mordida”), a penetração inicial ou a oviposição (inibição de oviposição, segundo alguns
500 autores); e deterrente, quando impede a continuidade da alimentação ou oviposição. A resposta
501 final do inseto depende do balanço entre os estímulos positivos e negativos como, por exemplo,
502 se o estímulo negativo repelente sobrepor ao atraente, o inseto não se orientará em direção à
503 planta e vice-versa (WHITTAKER; FEENEY, 1971).

504

505 **2.7.3 Antibiose**

506 Antibiose é o tipo de resistência preponderante nas plantas quando o inseto se alimenta
507 e ela exerce efeito adverso sobre a sua biologia, afetando direta ou indiretamente seu potencial
508 biótico (LARA, 1991; BALDIN et al., 2019). Os efeitos negativos nos insetos podem ser devido
509 às toxinas que as plantas apresentam como, por exemplo, a presença de inibidores de
510 crescimento ou reprodução, nutrição inapropriada que causam mortalidade nas fases de larva,
511 ninfa, pupa ou na muda, redução do tamanho e peso dos indivíduos e da fecundidade, alteração
512 da proporção sexual e da longevidade, entre outros parâmetros biológicos (BALDIN et al.,
513 2019).

514 O efeito adverso que a planta exerce sobre parâmetros da biologia do inseto e que faz
515 com que a planta apresente antibiose pode ser devido à presença de compostos antibióticos que
516 atuam na fisiologia e biologia do inseto, e podem ser: metabólitos tóxicos que atuam produzindo
517 intoxicação crônica ou aguda nos insetos, como alcaloides, glicosídeos cianogênicos e
518 cardíacos, quinonas, etc (BALDIN et al., 2019; VENDRAMIM, et al., 2019; antimetabólitos
519 que têm a propriedade de tornar os nutrientes essenciais indisponíveis aos insetos ou atuar como
520 inibidores enzimáticos; enzimas que atuam no processo de utilização do alimento, inibindo ou
521 reduzindo o processo normal da digestão; fitoesteroides como por exemplo alguns triterpenos,
522 saponinas e tetranortriterpenoides, como a azadiractina, que podem agir no inseto como seu
523 próprio hormônio juvenil, ou como hormônio da ecdisse, que induzem a metamorfose precoce,
524 esterilidade e diapausa devido ao desequilíbrio hormonal (VENDRAMIM et al., 2019).

525
526

527 **2.7.4 Tolerância**

528
529 Tolerância ocorre quando a planta resistente mesmo atacada da mesma forma que as
530 outras plantas suscetíveis, ela consegue se compensar, crescer e produzir normalmente. A
531 tolerância é caracterizada pela presença de atributos que não interferem sobre o inseto nem sua
532 biologia e nem no seu comportamento, sendo uma característica que faz com que da planta
533 sofra menos com o ataque do que outras em igualdade de condições. Muitas plantas podem
534 suportar uma determinada relação de injúrias sem ter perda de rendimento (PETERSON et al.,
535 2017; ERB, 2018; SPEROTTO et al., 2018). Desta forma, insetos-praga não são selecionados
536 quanto à resistência devido a ausência de pressão de seleção imposta por plantas tolerantes
537 (KOCH et al., 2019).

538 A tolerância, apesar da sua importância, tem sido menos utilizada e estudada do que a
539 antibiose e antixenose. Isto pode ser devido a vários fatores como, por exemplo, a grande
540 influência ambiental tornando mais difícil a identificação e seleção de plantas resistentes
541 (STOUT, 2013). Também, os mecanismos da tolerância ainda são pouco compreendidos,
542 complexos e de herança poligênica, tornando o processo mais lento. Por outro lado, o interesse
543 das empresas e dos produtores que utilizam as variedades resistentes é reduzir a incidência da
544 praga ao invés de gerenciar a biologia das plantas (PETERSON et al., 2017; ERB, 2018;
545 SPEROTTO et al., 2018). E mesmo que os insetos não tenham alterações biológicas e/ou
546 comportamentais, o entendimento básico de como as plantas lidam com a herbivoria, e a
547 identificação de genótipos tolerantes nas principais culturas podem ter um grande impacto no
548 manejo de pragas e rendimentos de produção.

549 Lembrando que a resposta das plantas à injúria da intensidade, do tempo, do tipo e da
550 parte da planta que foi injuriada e as interações com fatores ambientais (PETERSON; HIGLEY,
551 2001). A tolerância de plantas a insetos sugadores está principalmente associada a mecanismos
552 moleculares como desintoxicação e alterações na atividade fotossintética, enquanto os
553 mecanismos de tolerância em resposta a insetos mastigadores são geralmente descritos por
554 sobrecompensação através da produção de novos tecidos, realocação de recursos e mudança na
555 arquitetura da planta (TRUMBLE et al., 1993; STOWE et al., 2000; KRIMMEL; PEARSE,
556 2016).

557 Peterson et al. (2017) lista três fatores que restringem o desenvolvimento de
558 conhecimentos sobre a tolerância: falta de técnicas adequadas para identificar e incorporar a
559 tolerância para as culturas; a capacidade de cultivares tolerantes servirem como reservatórios
560 para vetores de vírus, por exemplo, e a falta de informações básicas sobre a herança da

561 tolerância. Para isto, devem-se realizar pesquisas básicas sobre os mecanismos fisiológicos e
562 bioquímicos de tolerância, a qual deve envolver pesquisa interdisciplinar entre biólogos,
563 ecologistas, químicos, biologia molecular, melhoristas de plantas entomologistas para utilizar
564 cada vez mais esta importante ferramenta no MIP.

565

566 **3 CONCLUSÃO**

567 Concui-se que devendo a importância da cultura do sorgo, é recomendado integrar o
568 máximo possível de estratégias de manejo de pragas, intensificando a necessidade de buscar
569 táticas eficientes de manejo, como por exemplo, a resistência de plantas. Diante de todas as
570 ferramentas disponíveis para trabalhar com resistência de plantas, surge a necessidade de mais
571 estudos em relação a resistência do sorgo às suas principais pragas. Devido ao tempo em que
572 os principais trabalhos foram realizados com resistência de sorgo, principalmente, apenas com
573 sorgo granífero, aliado à falta de trabalhos com sorgo de diferentes categorias, deve-se diante
574 das novas técnicas e perspectivas de pesquisa em resistência de plantas a insetos, juntamente
575 com o crescimento da importância do sorgo no país, realizar mais estudos em programas de
576 melhoramento de sorgo, compatibilizando a maior produtividade e a resistência às principais
577 pragas.

578

579

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SEGUNDA PARTE – ARTIGOS

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ARTICLE 1- Resistance of *bmr* energy sorghum hybrids to sugarcane borer and fall armyworm

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987 **Abstract**

988 The lower lignin content in plants species with energy potential results in easier
989 cellulose breakdown, making glucose available for ethanol generation. However, higher lignin
990 levels can increase resistance to insect attack. The objective of this work was to evaluate the
991 susceptibility of a *bmr*-6 biomass sorghum (a mutant genotype with a lower concentration of
992 lignin) to important pests of sorghum energy, *Diatraea saccharalis* and *Spodoptera frugiperda*.
993 Experiments were performed in the laboratory and greenhouse to evaluate the development of
994 these pests on the biomass sorghum *bmr* hybrids BR007, BR008, and TX635 and their
995 respective conventional near-isogenic genotypes (without the *bmr* gene). The lignin content
996 was higher in non-*bmr* hybrids, but in the parameters evaluated in insects, there was variation
997 between treatments, but not in relation to being *bmr* or not. The lowest survival of *S. frugiperda*
998 was observed in the BR008 hybrid, both *bmr* and non-*bmr*. The *S. frugiperda* injury scores on
999 plants in the greenhouse were high (>7) in all treatments. For *D. saccharalis*, there was no
1000 difference in larval survival in the laboratory, but in the greenhouse, the BR007 hybrid, both
1001 *bmr* and non-*bmr*, provided greater survival. Due the need to diversify the energy matrix and
1002 the fact that greater susceptibility of the *bmr* hybrids to either pests was not found in this study,
1003 these results hold promise for cultivation of these biomass sorghum hybrids for the production
1004 of biofuels.

1005

1006 **Keywords:** plant resistance, energy sorghum, *Diatraea saccharalis*, *Spodoptera*
1007 *frugiperda*

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1012 **Resistência de híbridos de sorgo energia *bmr* à broca da cana-de-açúcar e à lagarta**
1013 **do cartucho**

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1015 **Resumo**

1016 O menor teor de lignina em espécies de plantas com potencial energético resulta na maior
1017 facilidade de quebra da celulose, disponibilizando glicose para geração de etanol. Porém,
1018 maiores teores de lignina representam um fator de resistência ao ataque de insetos-praga. O
1019 objetivo deste trabalho foi avaliar a suscetibilidade de um sorgo biomassa *bmr*-6 (um genótipo
1020 mutante com menor concentração de lignina) à importantes pragas do sorgo energia, *Diatraea*
1021 *saccharalis* e *Spodoptera frugiperda*. Foram realizados experimentos em laboratório e casa de
1022 vegetação, avaliando o desenvolvimento destas pragas nos híbridos de sorgo biomassa *bmr* 007,
1023 008, TX635 e seus respectivos genótipos isogênicos convencionais (sem o gene *bmr*). O teor
1024 de lignina foi maior nos híbridos não *bmr*, mas nos parâmetros avaliados nos insetos, houve
1025 variação entre os tratamentos, mas não em relação a ser ou não *bmr*. A menor sobrevivência de
1026 *S. frugiperda* foi verificada no híbrido BR008 tanto *bmr* quanto não *bmr*. As notas de injúria
1027 por *S. frugiperda* no sorgo em casa de vegetação foram altas (>7) em todos os tratamentos. Para
1028 *D. saccharalis*, não houve diferença significativa para a sobrevivência larval em laboratório,
1029 mas em casa de vegetação o híbrido BR007 tanto *bmr* quanto não *bmr* proporcionaram maior
1030 sobrevivência. Diante da necessidade de diversificar a matriz energética e o fato de que não foi
1031 comprovada neste estudo maior suscetibilidade dos híbridos *bmr* a ambas as pragas, estes
1032 resultados são promissores para o cultivo desses híbridos de sorgo biomassa para produção de
1033 biocombustíveis.

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1035 **Palavras-chave:** Resistência de plantas, sorgo energia, *Diatraea saccharalis*,
1036 *Spodoptera frugiperda*.

1037 **Introduction**

1038 One of the impediments to the conversion of biomass into biofuels is the presence of the
1039 polymer lignin, which interferes with the release of sugars from the cell wall polysaccharides
1040 cellulose and hemicellulose during enzymatic saccharification (Rubin, 2008; Dien et al., 2009).
1041 Despite the increase in use of starch- and sugarcane-based biofuels, the fuels produced from
1042 lignocellulosic biomass are greenhouse-gas-favorable alternative energy sources (Rubin, 2008).
1043 Lignocellulosic biomass constitutes the residues of plants, such as elephant grass, coconut husk,
1044 and biomass sorghum, which do not have the sugar contents found in sugarcane and sweet
1045 sorghum (Santos et al., 2011; Hernández et al., 2015).

1046 In Brazil, there has been a significant increase in export of electricity from biomass in
1047 last five years. The share of biomass sources out of the total composition of exported energy in
1048 the National Interconnected System (National Interconnected System) increased from 17% in
1049 2013 to 19% in 2018 (EPE, 2019). This increase in the demand for heat generation from
1050 biomass is due to its lower cost and higher practicality, since it is used directly through
1051 combustion in ovens and boilers. Another important factor is that burning of fossil fuels emits
1052 various contaminants that cause local, regional, and global environmental impacts. In the
1053 Nationally Determined Contribution (NDC), Brazil is committed to reducing greenhouse gas
1054 emissions by 43% by 2030 and increasing the share of sustainable bioenergy in the energy
1055 matrix to approximately 18% by expanding its biofuels consumption, by increasing the share
1056 of advanced biofuels, known as second-generation biofuels (EPE, 2019).

1057 Biomass sorghum (*Sorghum bicolor* L. Moench) is a promising lignocellulosic raw
1058 material because it is a productive crop adapted to water stress conditions, which is an
1059 advantage in a scenario that demands water savings, in addition to having a well-understood
1060 production system and being adapted to different environmental conditions (Silva et al., 2017).
1061 The biomass sorghum under suitable photoperiod conditions, has the potential to produce up to

1062 102.22 t ha⁻¹ of fresh biomass yield, its cultivation is completely mechanized, and the plants
1063 have calorific power similar to that of sugarcane, eucalyptus and elephant grass needed for
1064 burning, between 16 e 19 MJ·kg⁻¹ (May, 2013; Parrella, 2013). Sorghum biomass can be used
1065 as a raw material for bioenergy through the production of second-generation ethanol as a liquid
1066 biofuel, and in energy generation by direct biomass burning, as well as food for ruminants
1067 (Cherney et al., 1991 Zegada-Lizarazu and Monti, 2012; Reddy and Blummel, 2020). The
1068 *brown midrib (bmr)* mutant of biomass sorghum is an alternative in energy sector for use in
1069 processes in which lignin is an obstacle, such as the production of second-generation ethanol.
1070 The simplification of saccharification process is very important because the cost of cellulases
1071 to degrade biomass is a limiting factor for the economic production of biofuels. For this, the
1072 raw material needs to have a low lignin content, as the biomass sorghum *bmr*, because
1073 cellulose is a glucose polymer that has to be broken down, so a lower lignin content means
1074 easier cellulose breakage, making glucose available for ethanol generation (DIEN, et al., 2009).
1075 With high productivity and contribute to the strategy of a green economy with the supply of
1076 raw material in the distilleries, it is considered that sorghum biomass is a renewable and low-
1077 cost source of energy, being economically viable (Parrella, 2013; Vendruscolo et al., 2016). In
1078 addition, this mutant genotype may provide better digestibility to cattle because lignin is the
1079 nondigestible fraction of the plant that supports the stem. The higher the lignin content, the
1080 lower the silage quality and digestibility, as it is the factor that most limits the availability of
1081 cell wall components for bovine rumen microorganisms (Reddy and Blummel, 2020). The
1082 lignin, cross-links cellulose and can be considered as “cell glue” that gives resistance to plant
1083 tissue and gives rigidity to the cell wall, thus factors such as the incidence of pests can make
1084 the plant even more fragile (Rubin, 2008).

1085 The occurrence of pest insects is a problem for sorghum crops, and the sugarcane borer
1086 *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) is one of the major pests. The

1087 greatest injury sugarcane borers can cause to a plant that can reach five meters in height is to
1088 make the stem fragile and susceptible to lodging, in addition to hindering the flow of sap and
1089 photoassimilates in the plant (Mendes et al., 2014; Silva et al., 2017). The fall armyworm,
1090 *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), is also one of the main
1091 sorghum pests, causing defoliation and thereby reducing leaf area for photosynthesis. The
1092 presence of higher lignin content in plants can be a resistance trait to insect pests, especially
1093 lepidopterans (Dow et al., 2016). Thus, the absence of this characteristic could reduce the
1094 natural morphological barrier of plants and increase their susceptibility to pest infestation. This
1095 possibility has not been fully elucidated in sorghum and results vary widely between studies
1096 (Dowd et al., 2015, 2016).

1097 This study evaluated the susceptibility of bmr-6 sorghum to *D. saccharalis* and *S.*
1098 *frugiperda*, if the lower lignin content in the mutant plants favors feeding and pest performance.
1099 Knowledge of this information is highly important, since reducing the lignin content in the
1100 bioenergy feedstock could cause greater susceptibility to pest attack because this phenolic
1101 compound overall represents a plant defense trait against herbivorous insects (Dowd et al. 2016;
1102 Vendramim and Guzzo, 2009). Plants have chemical mechanisms for defense against insects,
1103 such as nitrogen compounds, terpenoids and phenolics. These compounds can be toxic to
1104 insects, preventing or altering their normal development (Vendramim et al., 2019). Lignin is
1105 made up of three main phenolic components: p-coumaryl alcohol (H), coniferyl alcohol (G) and
1106 synapyl alcohol (S), are aromatic polymers that vary in their branches and condense into
1107 different structures (Rubin, 2008). Therefore, the objective of this work was to evaluate the
1108 susceptibility of a *bmr-6* biomass sorghum (a mutant genotype with a lower concentration of
1109 lignin) to important pests of sorghum energy, *Diatraea saccharalis* and *Spodoptera frugiperda*.
1110
1111

1112 **Material and Methods**

1113

1114 **Experimental site and conditions**

1115

1116 Experiments with both pest species were carried out in a greenhouse and at the
1117 Laboratory of Ecotoxicology and Insect Management of Embrapa Milho e Sorgo located in
1118 Sete Lagoas, Minas Gerais state, Brazil, in a climate-controlled room with 25 ± 2 °C
1119 temperature, 12-hour photoperiod, and $60 \pm 10\%$ relative humidity.

1120

1121 **Effect of the presence of the *bmr* gene on the development of sugarcane borer**

1122

1123 The biomass sorghum *bmr* hybrids BR007, BR008, and TX635 and their near-isogenic
1124 genotypes (without the *bmr* gene) were evaluated in a laboratory bioassay with six genotypes
1125 (=treatments). To obtain the leaves for feeding the insects, the hybrids were grown in the field,
1126 the soil in the region is of the red yellow latosol, with medium and silty texture (Embrapa,
1127 2013). The design of the experiment was in randomized blocks, with four replications, each
1128 experimental plot consisting of three lines of 5 m in length and 0.7 m in spacing. The soil was
1129 fertilized with 400 kg ha^{-1} of NPK 8-28-16, and at 15 days after emergence, cover fertilization
1130 was performed using 200 kg ha^{-1} of urea. The plants were thinned 15 days after emergence,
1131 leaving eight plants per meter, in a total of 40 plants per row in plots. Management practices
1132 performed according to May (2013), except that no insecticides were sprayed in the
1133 experimental area.

1134 Testing insects were obtained from a rearing colony in the laboratory. Briefly, larvae
1135 were individually reared on artificial diet based on cooked beans, wheat germ, and casein
1136 (Bowling, 1967). Adults were transferred to cylindrical mating cages (40 cm h x 30 cm in diam.)

1137 containing moth food (10% sugar and 5% ascorbic acid in water) and white sulfite paper on the
1138 inner walls for oviposition. Collected egg masses were let to hatch, and neonates transferred to
1139 the artificial diet (Cruz, 2000). Newly hatched larvae obtained from the laboratory rearing
1140 colony were individually placed in 50-mL plastic cups sealed with acrylic lids, according to the
1141 method adapted from Mendes et al. (2011) for *S. frugiperda*.

1142 Whorl leaves from the *bmr* and non-*bmr* sorghum were collected from the plants when
1143 there were between six and eight fully developed leaves (stages V6-V8) (Magalhães and
1144 Durães, 2003) and taken to the laboratory, where they were cleaned and cut into pieces of
1145 approximately 50 cm². The leaves in the bioassay containers were replaced every 48 hours.

1146 The survival and biomass of *D. saccharalis* larvae were evaluated 10 days after the
1147 beginning of the experiment. The evaluation was performed for 10 days, which is the duration
1148 of the behavior of *D. saccharalis* in seeking the plant stem; after that, the larvae no longer feed
1149 on the leaves. Data on these biological parameters were subjected to the Shapiro-Wilk and
1150 Bartlett tests to check the assumptions of normality of residuals and homoscedasticity,
1151 respectively. They were then subjected to analysis of variance (ANOVA), and means of
1152 treatments were compared by Tukey's test ($\alpha=0.05$).

1153 A second experiment was conducted in a greenhouse with the three biomass sorghum
1154 *bmr* hybrids (BR007, BR008, TX635) and their near-isogenic genotypes (without the *bmr*
1155 gene). The hybrids were planted in a completely randomized design to evaluate the resistance
1156 (antixenosis/antibiosis) to sugarcane borer. Planting was performed in 20-L pots filled with soil
1157 fertilized with 50 g of 08-28-16 NPK and 0.3% Zn/100 kg·v. For each treatment, 20 pots with
1158 three plants were used, and each pot was considered a replicate. At the four-to-six-developed
1159 leaf stage (Magalhães and Durães, 2003), the plants were infested with five newly hatched *D.*
1160 *saccharalis* larvae per plant, totaling 15 larvae per pot. Injury caused by bored larvae was
1161 evaluated every 60 days after infestation.

1162 For injury evaluation, plants were cut close to the ground and opened longitudinally to
1163 detect the presence of galleries bored in the stem of plants. The parameters evaluated were plant
1164 height (cm), bored internodes (%), gallery size (cm), number of galleries per plant, survival (%)
1165 and biomass (mg) of larvae and pupae recovered from the plants. Data recorded for these
1166 parameters were subjected to the Shapiro-Wilk and Bartlett tests to check the assumptions of
1167 normality of residuals and homoscedasticity, respectively, and then analyzed by ANOVA. The
1168 means of treatments were compared by Tukey's test ($\alpha=0.05$). The analyses were performed
1169 using the statistical R software version 3.5.3 (R Development Core Team 2019).

1170

1171 **Effect of the presence of the *bmr* gene on the development of fall armyworm**

1172

1173 This experiment was carried out in the same manner as described for the sugarcane
1174 borer. The variables evaluated in *S. frugiperda* were larva-to-adult survival (%), biomass (mg)
1175 of larvae at 10 days, and biomass (mg) of pupae at 48 hours. To evaluate survival, a group of
1176 10 individuals was considered one replicate, and there were nine replicates (90 individuals) in
1177 the experiment. For the other biological variables, one individual was considered a replicate.
1178 Because mortality was different in each treatment, the number of individuals (replicates)
1179 available for statistical analysis varied.

1180 The adaptation index (AI) proposed by Boregas et al. (2013) was used to evaluate the
1181 larval performance of *S. frugiperda* whereby: $AI = \text{larval survival (\%)} \times \text{pupal biomass}$
1182 (mg)/larval development period (days); in the calculation of AI, pupal biomass was used to
1183 estimate the fecundity of adults (Barah and Sengupta, 1991). Correlation coefficients were also
1184 estimated to correlate the biological variables with the AI of *S. frugiperda*.

1185 For the survival analysis, a curve was generated in SigmaPlot software 10.0[®] (Systat
1186 Software Inc.) from the output of the chi-squared test. For the larval and pupal biomass data,

1187 the Shapiro-Wilk test was performed to check normality and the Bartlett test was used to check
1188 for homogeneity of variances. As data did not follow a normal distribution or exhibited
1189 heterogeneity of variances, was performed using generalized linear model (GLM) and negative
1190 binomial distribution, and the means of treatments were compared by Tukey's test ($\alpha=0.05$).
1191 The analyses were performed using the statistical R software version 3.5.3 (R Development
1192 Core Team 2019).

1193 The experiment to evaluate plant injury in the greenhouse followed the same procedure
1194 described for the sugarcane borer. Seven replicates were used per treatment, totaling 42 potted
1195 plants. Plants at the four-to-six-developed leaf stage were infested with five newly hatched *S.*
1196 *frugiperda* larvae per plant, totaling 15 larvae per pot. The pots were covered with a voile fabric
1197 cage (1.20 cm \times 55 cm) to prevent the larvae from escaping.

1198 Evaluation of plant injury was made through visual injury scores according to the scale
1199 proposed by Davis and Williams (1992) for corn and adapted to sorghum. Evaluations were
1200 performed at 7, 14, and 21 days after larval infestation. The scores assigned to the plants ranged
1201 from 0 to 9, as follows: 0 = no injury; 1 = presence of pinholes (more than one pinhole per
1202 plant); 2 = pinholes and one to three small circular lesions (up to 1.5 cm); 3 = one to five small
1203 circular lesions (up to 1.5 cm), plus one to three elongated lesions (up to 1.5 cm); 4 = one to
1204 five small circular lesions (up to 1.5 cm), plus one to three elongated lesions (> 1.5 cm and <
1205 3.0 cm); 5 = one to three large elongated lesions (>3 cm) in one to two leaves, plus one to five
1206 holes or elongated lesions (up to 1.5 cm); 6 = one to three large elongated lesions (>3 cm) in
1207 two or more leaves, plus one to three large holes (>1.5 cm) in two or more leaves; 7 = three to
1208 five large elongated lesions (> 3.5 cm) in two or more leaves, plus one to three large holes
1209 (greater than 1.5 cm) in two or more leaves; 8 = many elongated lesions (> 5 cm) of all sizes in
1210 most leaves, many medium to large holes (> five) larger than 3 cm in many leaves; 9 = almost

1211 completely destroyed leaves. The injury scores were analyzed by calculating their confidence
1212 intervals at 95% probability.

1213

1214 **Bromatological analyses**

1215

1216 Bromatological analyses were performed in the sorghum hybrids to identify possible
1217 differences in chemical composition between *bmr* and non-*bmr* genotypes. To determine the
1218 dry matter mass, the plants were placed in paper bags and dried in an oven at 65 °C for 72 hours.
1219 The samples were milled in a knife mill with a 2-mm sieve (Wiley mill, Arthur H. Thomas,
1220 Philadelphia, PA, USA) and prepared for chemical analysis.

1221 The contents of acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid
1222 detergent lignin (ADL) were determined according to the method proposed by Robertson and
1223 Van Soest (1981). The cellulose content was calculated as the difference between the ADF and
1224 lignin contents, and the hemicellulose content was calculated as the difference between NDF
1225 and ADF by near-infrared (NIR) spectroscopy (NIRFlex 500, Buchi Brasil Ltda, Valinhos, SP,
1226 Brazil). The calibration equations for the analysis of ADF, NDF, lignin, and calorific value
1227 were based on values obtained and stored in the Embrapa Corn and Sorghum database, which
1228 covers a total of 400 samples.

1229 Data obtained from the chemical analyses were subjected to the Shapiro-Wilk and
1230 Bartlett tests to check the assumptions of normality of residuals and homoscedasticity,
1231 respectively, and were analyzed by ANOVA. The means of treatments were compared by
1232 Tukey's test ($\alpha=0.05$). The analyses were performed using the statistical R software version
1233 3.5.3 (R Development Core Team 2019).

1234

1235

1236 **Results**

1237

1238 **Effect of the presence of the *bmr* gene on the development of sugarcane borer**

1239

1240 There was no difference in larval survival of *D. saccharalis* among the energy sorghum
1241 hybrids. Sugarcane borer larvae fed the *bmr* hybrids showed mean 10-day-old biomass 22%
1242 higher than larvae reared in the non-*bmr* near-isogenic genotypes (Table 1).

1243 The plant height, total number of internodes, number of healthy and bored internodes,
1244 and length and diameter of galleries, had no significant differences. The BR007 hybrid, both
1245 *bmr* and non-*bmr*, provided higher percentage survival of *D. saccharalis* than the other hybrids.
1246 The TX635 and BR007 hybrids had the highest and lowest mean pupal biomass, respectively
1247 (Table 2).

1248

1249 **Effect of the presence of the *bmr* gene on the development of fall armyworm**

1250

1251 There was difference in larval survival of *S. frugiperda* among the sorghum hybrids.
1252 TX635 had the highest percentage survival, followed by BR007 *bmr*, BR007, and TX635 *bmr*.
1253 The lowest larval survival was observed in the BR008 hybrid, both in the *bmr* genotype and
1254 conventional near-isogenic genotype (Fig 1).

1255 Larval biomass differed between hybrids, with higher biomass in BR007 *bmr*, followed
1256 by TX635; the lowest biomass of fall armyworm was obsered in BR008, both *bmr* and non-
1257 *bmr*. The biomass of *S. frugiperda* pupae was greater in the BR008 hybrid than in the other
1258 sorghum hybrids (Fig 2).

1259 The AI of *S. frugiperda* was 11.64 in the TX635 hybrid, 10.08 in TX635 *bmr*, 11.36 in
1260 BR007, 10.45 in BR007 *bmr*, 10.01 in BR008, and 9.07 in BR008 *bmr*, without difference

1261 between means. The estimated correlation coefficient between the AI and larval survival was
1262 0.875; between AI and pupal biomass, -0.390; and between AI and larval biomass, -0.877.

1263 The injury scores of *S. frugiperda* in sorghum plants differed among sorghum hybrids.
1264 The TX635 hybrid, both *bmr* and non-*bmr*, had a lower injury score at 7 days than at 14 or 21
1265 days after infestation, as did the BR007 *bmr* hybrid. The other hybrids did not differ across the
1266 evaluated days. The highest injury scores were found in the TX635 *bmr* and non-*bmr* hybrids,
1267 with the highest score (9) observed in the last evaluation date (Fig 3).

1268

1269 **Bromatological analyses**

1270

1271 The levels (%) of ADF, NDF, and hemicellulose had no differences among the energy
1272 sorghum hybrids. The dry matter was highest in the BR007 non-*bmr* hybrid and lowest in the
1273 TX635 non-*bmr* hybrid. Lignin percentage was higher in the non-*bmr* hybrids than in the
1274 conventional near-isogenic genotypes. Finally, the calorific value (MJ/kg) was highest in the
1275 BR008 non-*bmr* hybrid and lowest in TX635 *bmr* (Table 3).

1276

1277 **Discussion**

1278

1279 The hypothesis of greater susceptibility to attack of sugarcane borer and fall armyworm
1280 due to the lower lignin content in *bmr*-6 sorghum hybrids was not supported by our findings.
1281 Although microorganisms that can degrade cellulose, hemicellulose, and lignin have been
1282 identified in the midgut of *D. saccharalis* larvae, they have a greater capacity to digest cellulose
1283 than hemicellulose, and few produce enzymes to degrade lignin (Dantur, 2015). Thus, plant
1284 genotypes with lower lignin levels, in theory, could be more consumed by insect pests because
1285 lignin is difficult to digest. Our results showed that *D. saccharalis* larval biomass was higher in

1286 *bmr* than in non-*bmr* sorghum hybrids. This finding demonstrates the effect of plant biomass
1287 on insect development, as the greater the biomass, the greater the insect growth rate is,
1288 indicating that the host plant is suitable for herbivore development and does not show resistance
1289 (Souza et al., 2019). It is possible that the *bmr* hybrids are more suitable for the development
1290 of *D. saccharalis*, which harbors microorganisms capable of digesting lignin, though our
1291 survival data do not show such a trend.

1292 The height, total number of internodes, number of healthy and bored internodes, and
1293 length and diameter of galleries made by *D. saccharalis* did not show significant differences
1294 between *bmr* and non-*bmr* genotypes or among sorghum hybrids. The BR007 hybrid, both *bmr*
1295 and non-*bmr*, caused higher *D. saccharalis* percentage survival than the other hybrids; TX635
1296 provided greater pupal biomass; and the non-*bmr* BR007 hybrid, lower pupal biomass. Thus,
1297 there was no consistency in the results that would show an effect of the *bmr* gene on *D.*
1298 *saccharalis*. Again, the results of the greenhouse experiment did not indicate higher levels of
1299 resistance in the energy sorghum *bmr* genotypes relative to the non-*bmr* genotypes.

1300 Higher survival of *S. frugiperda* larvae was found in the TX635 hybrid than in the
1301 respective near-isogenic *bmr* genotype under laboratory conditions. This hybrid had a greater
1302 difference in lignin content between the *bmr* and non-*bmr* genotypes, and there may also be
1303 other causes of resistance involved besides this trait, which are still unknown. The leaves of the
1304 *bmr* genotypes had lower lignin contents, as shown by the results of the bromatological analysis,
1305 which was expected. Lower lignin contents may make plants more susceptible to herbivory,
1306 since lignin is an important chemical and morphological component of plant resistance that can
1307 hinder larval feeding (Dowd et al., 2016). However, the BR008 hybrid, both *bmr* and non-*bmr*,
1308 caused lower larval survival and biomass of *S. frugiperda*, which did not differ. Thus, the
1309 presence or absence of the *bmr* gene did not alter the larval performance. These results indicate
1310 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

1336 and observed a greater effect of harvest than of the *bmr* mutation. Additionally, Dowd and
1337 Satller (2015) found no differences in *S. frugiperda* mortality in *bmr* sorghum. The authors
1338 suggested the presence of the brown midrib in leaves of *bmr* sorghum may, contrary to
1339 expectations, increase the resistance of the plants, since they may be less nutritionally suitable
1340 for larvae because of this trait and possibly because of other chemical and morphological
1341 changes related to the presence of the *bmr* mutation. Our results also suggest this conclusion
1342 because although no microorganism that can digest lignin was found in the midgut of *S.*
1343 *frugiperda* (Dantur 2015), the *bmr* mutation had no effect on fall armyworm.

1344 Regarding the *S. frugiperda* injury scores in sorghum hybrids in the greenhouse, no
1345 differences were observed between the *bmr* and non-*bmr* genotypes. The highest injury scores
1346 were found in the TX635 *bmr* and non-*bmr* hybrids, with maximum scores (9) observed at 14
1347 and 21 days of larval infestation. The hybrids studied herein had the *bmr*-6 mutation, which
1348 causes reduced cinnamyl alcohol dehydrogenase activity (Oliver et al., 2005). When evaluating
1349 *bmr*-6 and *bmr*-12 sorghum hybrids, Dowd et al. (2016) did not detect consistent susceptibility
1350 to *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) or *S. frugiperda* in any *bmr*
1351 genotype compared to the susceptibility of the nonmutant isogenic genotypes. However, those
1352 authors reported evidence of increased resistance in the *bmr*-6 genotypes compared to the near-
1353 isogenic genotypes, and greater susceptibility of the *bmr*-12 plants to the insects both in the
1354 field and in laboratory. The results obtained by Dowd et al. (2016) differ from the results found
1355 herein, as we did not consistently find increased resistance or susceptibility of the *bmr* gene to
1356 the sugarcane borer and fall armyworm. This subject is still not resolved given the varying
1357 responses found in the literature.

1358 Plant dry matter was highest in the BR007 non-*bmr* hybrid and lowest in the TX635
1359 non-*bmr* hybrid. Thus, the BR007 genotype was more productive than the *bmr* genotype, which
1360 may contribute to a higher tolerance to pest infestation relative to the other two hybrids.

1361 Conversely, the calorific value was highest in the BR008 non-*bmr* hybrid and lowest in TX635
1362 *bmr*. This trait of higher energy content is important for energy generated from direct burning
1363 of biomass (Parrela et al., 2013).

1364 Cellulose is the major structural component of plant cell walls; hemicellulose is the
1365 second most abundant component in lignocellulosic biomass; and lignin is the compound that
1366 gives greater rigidity to plant fibers and confers resistance to insects and pathogens. However,
1367 this trait hinders biofuel production (Dien et al., 2009; Rubin, 2008; Del Rio et al., 2007; Van
1368 Wyk, 2001). Although a reduction in lignin concentration was detected in the *bmr* genotypes,
1369 there were no differences in ADF, NDF, or hemicellulose contents, which did not differ
1370 between the *bmr* and non-*bmr* sorghum hybrids. Ebling and Kung Jr. (2004) also found no
1371 differences in the percentage of NDF or ADF between *bmr* and non-*bmr* corn, but found
1372 differences in their lignin content. These findings corroborate the results found herein, where
1373 the percentage of lignin was higher in non-*bmr* sorghum hybrids; the TX635 hybrid had the
1374 highest lignin content; and the greatest difference in lignin content was found between the *bmr*
1375 and non-*bmr* genotypes.

1376 The parameters evaluated for *D. saccharalis* and *S. frugiperda* in our study varied
1377 between treatments, and were not consistent or predominant in only one energy sorghum hybrid
1378 or in *bmr* or non-*bmr* genotypes. From the viewpoint of integrated pest management, it is
1379 interesting that there is resistance to pests in non-*bmr* sorghum that is grown for other purposes
1380 than biomass, such as energy cogeneration through direct burning. However, for biofuel
1381 production, which is the main purpose of *bmr* sorghum plants due to its lower lignin content,
1382 the fact that it has no greater susceptibility to the main crop pests is highly important.

1383 We can conclude that cultivation of *bmr* energy sorghum can be safe within the context
1384 of integrated pest management because it is not more susceptible to the major crop pests,
1385 sugarcane borer and fall armyworm, and regardless of whether the sorghum is *bmr* or not,

1386 control measures, using chemical and biological approaches should be applied whenever the
1387 economic thresholds are attained. Given the need to diversify the energy matrix in Brazil and
1388 worldwide since renewable energy is a key source of energy security, provides reduced
1389 dependence on fossil fuels, and causes to lesser emission of greenhouse gases, the results of
1390 this work show that cultivation of these biomass sorghum hybrids holds great promise for
1391 biofuel production.

1392

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1532 **Table 1** Means (\pm SE) of larval survival (%) and larval biomass (mg) at 10 days of
 1533 *Diatraea saccharalis* in *bmr* and conventional non-*bmr* near-isogenic hybrids

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

1534 Means followed by different letters in the same column are different by Tukey's test (P
 1535 < 0.05).

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1549 **Table 2** Means (\pm SE) of plant height (cm), total number of internodes, number of
 1550 healthy and bored internodes, gallery length and diameter (cm), and survival (%) and biomass
 1551 (mg) of *Diatraea saccharalis* pupae in *bmr* sorghum hybrids and non-*bmr* near-isogenic
 1552 genotypes

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

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1554 Means followed by different letters in the same column differ according to Tukey's test
 1555 ($P < 0.05$).

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1559 **Table 3** Means (\pm SE) of variables of the bromatological analysis of *bmr* and non-*bmr*
 1560 sorghum hybrids

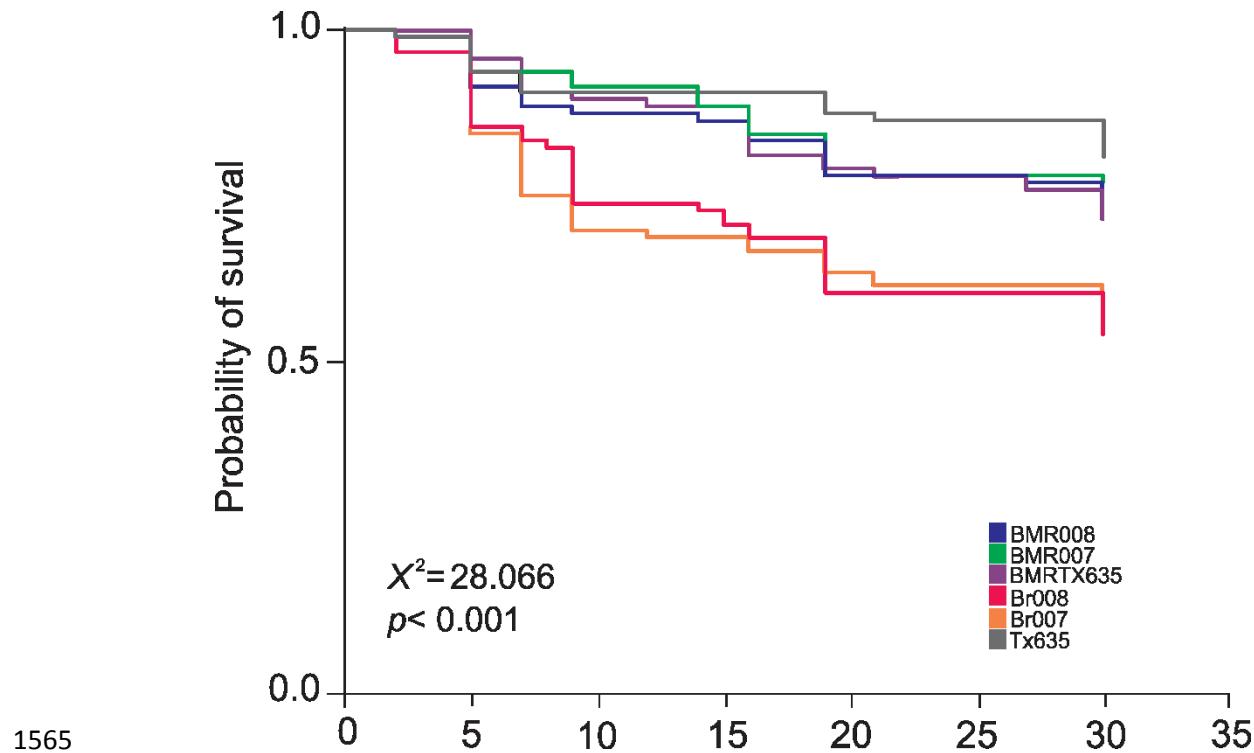
VARIABLE	HYBRID	<i>Bmr</i>	non- <i>bmr</i>
Dry matter 65 °C (MG ha ⁻¹)	TX635	14.23 \pm 0.03 ab	11.48 \pm 0.80 b
	BR007	14.13 \pm 0.46 ab	17.42 \pm 2.11 a
	BR008	12.87 \pm 1.25 ab	15.05 \pm 0.93 ab
ADF (%)	TX635	35.28 \pm 0.91 a	37.92 \pm 0.96 a
	BR007	33.88 \pm 2.13 a	36.65 \pm 3.06 a
	BR008	34.74 \pm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

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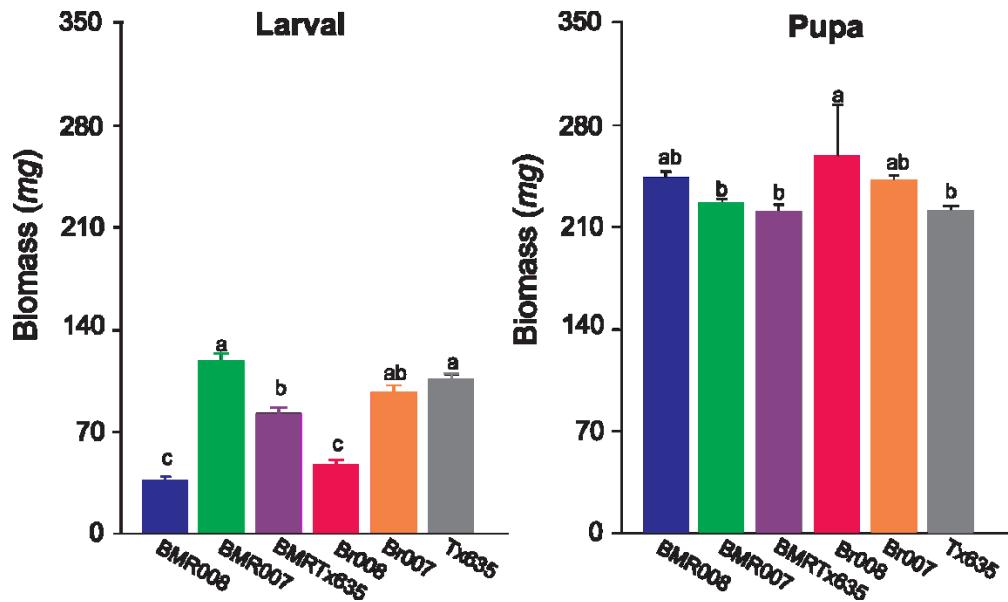
1562 Means followed by different letters in the same column differ according to Tukey's test

1563 (P < 0.05).

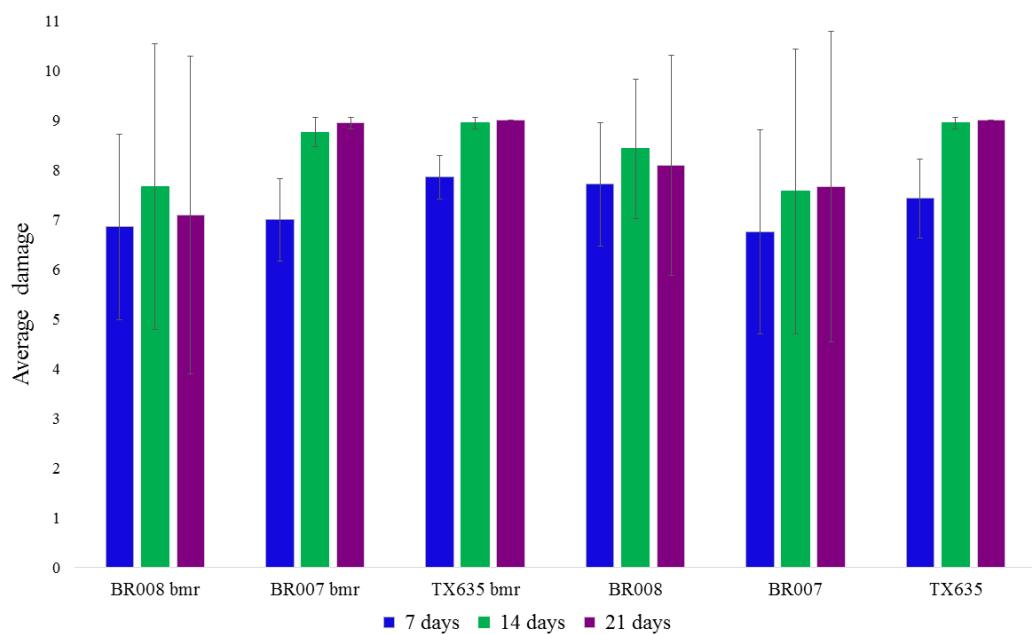
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1566 **Fig 1** Survival curve of *Spodoptera frugiperda* as a function of days of development in
1567 *bmr* sorghum hybrids and their respective conventional non-*bmr* isogenic genotypes.
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1573 **Fig 2** Response of *Spodoptera frugiperda* feeding on leaves of *bmr* sorghum hybrids
1574 and their respective isogenic genotypes: larval biomass (mg) and pupal biomass (mg). Data are
1575 means and standard errors. Means followed by different letters differ according to Tukey's test
1576 (P < 0.05).
1577



1578 **Fig 3** Injury scale (0-9) for *Spodoptera frugiperda* at 7, 14, and 21 days after infestation
1579 by recently hatched larvae in the different hybrids of *bmr* sorghum and their respective
1580 conventional isogenic genotypes. Intervals between adjacent bars do not differ from one another
1581 by the confidence interval (P < 0.05).

1582 **ARTICLE 2- What is the potential of the sugarcane borer in reducing sorghum grain production?**

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1584 **Preparado segundo a norma e submetido à “NEOTROPICAL ENTOMOLOGY”**

1585

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1600 **Abstract**

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1602 Pest attack is an important factor in the loss of sorghum productivity, and one of the main pests of grain sorghum
1603 is *Diatraea saccharalis*. Thus, the objective of this paper was to evaluate the influence of *D. saccharalis* infestation
1604 on grain production in commercial sorghum hybrids. For this, grain sorghum hybrids were planted in three crops
1605 seasons: March 2018 (2018/1) (CROP SEASON 1), September 2018 (2018/2) (CROP SEASON 2) and March
1606 2019 (2019/1) (CROP SEASON 3). Six treatments were used in the experiment, the three hybrids of grain sorghum
1607 and the same three hybrids with chemical treatment using the product Altacor® (150 ml ha⁻¹), under natural
1608 infestation, in four replications. The parameters evaluated were: length of galleries (cm), plant height (cm), length
1609 (cm) and weight (g) of panicles, infestation intensity and productivity. The intensity of infestation in the three
1610 crops season was higher when not treated with insecticide and consequently resulted in lower productivity.
1611 Regarding the difference between seasons, the 1st season planting had lower infestation intensity. The hybrid DKB
1612 590 had the lowest tolerance level, whereby with insecticide treatment production increased 1.5 times in crop
1613 season 1 (149.77 %). In crop season 2, hybrid BRS 373 exhibited the lowest increase in grain yield with control
1614 (29.79%), followed by AG1090 with 33.13%, and again DKB 590 (40.79%) with greater dependence on chemical
1615 control to increase yield. Crop season 3 obtained the lowest yield among the three crops, and hybrid AG1090
1616 responded better to chemical control with, yield increase of 105.90%, which is equivalent to more than double the
1617 yield when using chemical control, followed by DKB 590 with 54.26% which indicates more than half of the
1618 increase in production, and BRS 373 with 38.19%. This is the first study that effectively shows how much grain
1619 loss occurs by *D. saccharalis* attack in grain sorghum. The sugarcane borer was able to cause yield loss up to 1.5x
1620 when sorghum plants were not controlled by insecticide treatment in the less tolerant hybrid, and 50% in the more
1621 productive and tolerant hybrid.

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1623 **Keywords:** Infestation intensity; *Diatraea saccharalis*; yield. IPM

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1630 **Introduction**

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1632 Sorghum (*Sorghum bicolor* [L.] Moench) is an important cereal crop used as grain, forage, and energy.
1633 Grain sorghum make up 40-60% in monogastric animal feed, and has potential to replace corn in ruminant feed in
1634 the future and as it has 95% of the biological value of corn and has lower cost, which can reach 15-45% less than
1635 corn (Waquil et al. 2003; Miranda et al. 2015; Carvalho 2020). Grain sorghum is also one of the main sources of
1636 energy, proteins, vitamins, and minerals for millions of people in semiarid regions of Africa and Asia (Fao 1995;
1637 Queiroz et al. 2015). Sorghum flour can replace wheat flour in food preparation, such as bread, cakes, and pasta;
1638 due to the fact that it has advantageous characteristics such as neutral taste, light color, and does not contain gluten,
1639 sorghum flour has great potential to be used in the preparation of food products for celiac individuals (Ciacci et al.
1640 2007).

1641 Brazil stands out as the 9th producer of grain sorghum in the world, contributing ~10% of worldwide
1642 production (IBGE, 2020). In the 2019 crop season, nearly 2.5 million tons of sorghum grains were produced in
1643 Brazil, with the state of Goiás being the largest producer of the cereal, and responsible for 42.8% of total national
1644 production, followed by the state of Minas Gerais. The attack of pest insects is an important biotic factor affecting
1645 yield losses in sorghum crop, reducing its potential for grain production. Thus, studying the behavioral patterns of
1646 pest insect infestation in their host plants is necessary to understand insect preference and choice for the host plant,
1647 the respective plant response to pest injury, and ultimately the effects on crop production (Knolhoff and Heckel,
1648 2013).

1649 The sugarcane borer *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) is the a primary pest
1650 main pest of sugarcane and is very harmful to other important crops such as corn, sorghum, and rice. In sorghum,
1651 *D. saccharalis* is a problem not only in Brazil, but also in other regions of South America, Central America, the
1652 Caribbean, and South of USA, and among the Lepidoptera it is considered the primary pest most important pest
1653 (Fabricius, 1794) (Lepidoptera: Crambidae) (Legaspi et al. 1997; Hayden 2012; Mendes et al. 2014; Grimi et al.
1654 2018).

1655 The galleries caused by *D. saccharalis* larvae in the plant stem make this plant structure weakened,
1656 rendering the plants more vulnerable to other environmental factors. The attack of *D. saccharalis* causes stem rot
1657 by favoring the entry of pathogenic microorganisms, hindering photoassimilates translocation, shortening the stalk
1658 internodes and toppling, and overall weakening the plant. In some regions of Brazil, the damage by *D. saccharalis*
1659 may correspond to 30% of total grain production. *D. saccharalis* is a harder to detect pest, and farmers can only

1660 see the losses when it is too late, because they do not usually monitor the population of this insect species in the
1661 field (Mendes et al. 2012, 2014; Silva et al. 2014; Araujo 2017).

1662 Within an integrated pest management approach, it is very important to use different control tactics for
1663 the control of key pests in sorghum crops, such as *D. saccharalis* in order to reduce the damage caused to grain
1664 production (Mendes et al. 2014; Vilela et al. 2016). Plant resistance is one of the tactics recommended within the
1665 IPM precepts, since it presents as one of the main advantages its compatible integration with other control methods.
1666 Plants naturally possess differences in terms of susceptibility to insect pests, that is, a phenotype can present greater
1667 or lesser resistance to pest attack, which can be expressed in a constitutive or induced manner (Lara 1991;
1668 Gatehouse 2002; Bastos et al. 2015; Armstrong et al. 2016).

1669 For sugarcane, the level of economic damage is 3% of infestation intensity; however, an infestation
1670 intensity of 1% can cause yield reductions of up to 0.28% in alcohol production, 0.49% in sugar production, and
1671 1.5% in stalks production (Arrigoni 2002). In sorghum that is more resistant to the borer compared to sugarcane,
1672 an economic injury level of 4% borer infestation can be used (Vilela et al. 2014). In our study, the reason for
1673 choosing the tested hybrids was that AG1090, DKB 590, and BRS373 belong to the main seed producing
1674 companies in Brazil, and also because these hybrids were among the most planted in the 2017/2018 crop season
1675 (Menezes et al. 2018). However, to the best of our knowledge, there is no information about borer-resistance levels
1676 in these hybrids.

1677 Due to the feeding behavior in making galleries in plant stem, *D. saccharalis* larvae are protected within
1678 this structure, hindering the action of chemical control. Despite this cryptic behavior, it is often the method most
1679 used by farmers for borer control due to its practicality and culture of use. The number of insecticide active
1680 ingredients registered for use in sorghum crop in Brazil is very restrict, in which only three molecules and four
1681 commercial products are available: spinetoram, alpha-cypermethrin, and teflubenzuron (Agrofit 2021). In the
1682 present paper, the insecticide chlorantraniliprole (commercial product Altacor®) was chosen for use against *D.*
1683 *saccharalis*; although it not registered for sorghum, it is a product successfully used for borer control in sugarcane
1684 that is the main host plant of the pest, and studies are lacking for evaluation of borer control in the sorghum crop
1685 (Oliveira et al. 2020; Souza et al. 2020).

1686 In grain sorghum there are no records of yield losses in face of sugarcane borer attack. This is the first
1687 study that deals with yield loss estimate in function of borer injury intensity. Thus, the objective of this work was
1688 to evaluate sugarcane borer damage potential in three of the most used commercial sorghum hybrids, as well as
1689 how they vary in resistance levels to borer injury.

1690 **Materials and Methods**

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1692 **Experimental site and conditions**

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1694 The study was conducted in an experimental field at Embrapa Milho e Sorgo, in Sete Lagoas, MG, Brazil.

1695 The region is at 772m above sea level, and the climate in Sete Lagoas is tropical. According to Köppen's
1696 classification, the climate is classified as Aw. 21.5 °C (average temperature), and 1279 mm is the average annual
1697 rainfall. The Embrapa research institution is located at 19°28'S, 44°15'08''W, and 732 m. The soil in the region is
1698 of the red yellow latosol type, with medium texture and silty (Embrapa, 2013). As the region of Sete Lagoas has a
1699 history of high natural infestation of *D. saccharalis* (Vilela et al. 2017), it was not necessary to carry out artificial
1700 infestation in sorghum plants.

1701

1702 **Experiment conditions**

1703

1704 The experiment was carried out in three consecutive crop seasons in the same experimental area: March
1705 2018 (2018/1) (CROP SEASON 1) (winter season), September 2018 (2018/2) (CROP SEASON 2) (summer
1706 season) and March 2019 (2019/1) (CROP SEASON 3) (winter season). The soil was fertilized with 400 kg ha⁻¹ of
1707 NPK 8-28-16. At 20 days after germination, cover fertilization was performed using 200 kg ha⁻¹ of urea. Weed
1708 control was carried out using atrazine-based herbicide, at a dose of 1.5 kg a.i. per hectare. Cultural practices were
1709 performed according to May et al. (2014). The sorghum hybrids used in the experiment were AG1090, BRS373
1710 and DKB590; these hybrids were chosen because they belong to the main seed producing companies in the country
1711 and were the most planted in recent crop seasons (Menezes et al., 2018).

1712 Plant thinning was done 15 days after germination, leaving 10 plants per row meter, for a total of 50 plants
1713 per row in the plots. The experiment was designed in complete randomized blocks. Each experimental plot
1714 consisted of six rows with 5 m in length and 0.7 m in spacing. In total, four blocks were used as replicates and six
1715 treatments were evaluated, consisting of three grain sorghum hybrids with and without chemical control with the
1716 insecticide chlorantraniliprole (Altacor®, FMC Química do Brasil LTDA, Campinas, SP, Brazil) at the dose
1717 recommended by the manufacturer of 150 ml ha⁻¹. The insecticide was applied at the beginning of plant
1718 development, in three applications in total; the first application took place 15 days after plant germination, and the
1719 other two applications were spaced every 10 days.

1720 **Evaluation of *D. saccharalis* injury and production of sorghum hybrids**

1721

1722 At the beginning of plant maturation phase, panicles on the three central rows of the plots were bagged
1723 to prevent bird attacks. These rows were then harvested at the end of grain maturation for evaluation of yield
1724 parameters.

1725 The plants were cut close to the ground at harvest (~120 days after germination) and were longitudinally
1726 opened to detect and quantify injury by *D. saccharalis* larvae. The parameters evaluated were: the length of
1727 galleries (cm), plant height (cm), infestation intensity (%), and yield (kg ha^{-1}). Infestation intensity (I.I.) of
1728 sugarcane borer was calculated according to the formula: I.I. (%) = number of bored internodes /total number of
1729 internodes x 100. The weight (g) of panicles was determined on a scale with sensitivity of hundredths of a gram,
1730 and plant height was measured with a ruler (cm). Through the weight of grains and determination of the moisture
1731 content of the grains in each plot, yield was estimated in kg ha^{-1} , using the formula: $Mc = (100 - Ui) \times Mi/100 -$
1732 Ui . Where: Mc = corrected mass; Ui = initial humidity degree; Mi = initial mass; Uc = degree of humidity
1733 correction (13%).

1734

1735 **Statistical analysis**

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1737 Data obtained in the three crop seasons were submitted to the Shapiro-Wilk test to check for normality of
1738 errors and to the Bartlett test for homogeneity of variances. As data did not follow a normal distribution or
1739 exhibited heterogeneity of variances, they were analyzed using generalized linear models (GLM) with Quasi
1740 Poisson distribution, and the means of treatments were compared by Tukey's test ($\alpha=0.05$). Pearson correlation
1741 analysis was performed on data of infestation intensity and yield. The analyses were performed using the statistical
1742 R software version 3.5.3 (R Development Core Team 2019).

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1750 **Results**

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1752 The results indicated significant differences both between sorghum hybrids and in hybrids treated and not
1753 with insecticide (Figure 1). Crop seasons were analyzed separately, but were plotted in the same figure for easier
1754 visualization of results. The hybrids treated with insecticide in the three crop seasons showed greater plant height,
1755 smaller size of gallery by *D. saccharalis* larvae, and greater panicle length and weight (Figure 1). The hybrid
1756 AG1090 had no difference in plant height when treated or not with insecticide in crop season 1, while the other
1757 hybrids had lower height when not treated with insecticide ($F= 13.34$, $df= 5$, $P<0.001$). In crop season 2, hybrid
1758 BRS373 had the lowest plant height both with insecticide and without application ($F= 45.54$, $df= 5$, $P<0.001$). In
1759 crop season 3, the three hybrids had higher plant heights when treated with insecticide; in addition, hybrid BRS
1760 373 without insecticide was the hybrid with the lowest height of plants ($F= 54.91$, $df= 5$; $P<0.001$) (Figure 1).

1761 The size of bored galleries in the three crop seasons was higher when sorghum hybrids were not treated
1762 with insecticide. In crop season 1 ($F= 11.58$, $df= 5$, $P<0.001$) e 3 ($F= 15.09$, $df= 5$; $P<0.001$) hybrids BRS373,
1763 AG1090, and DKB 590 had smaller gallery sizes in this order when treated with insecticide. In crop season 2, the
1764 three hybrids treated with insecticide showed smaller gallery size than untreated plants, but the hybrids did not
1765 differ ($F= 15.62$, $df= 5$, $P<0.001$) (Figure 1).

1766 Panicles size were greater when the hybrids when treated with insecticide. In crop season 1, insecticide-
1767 treated hybrids did not differ, showing larger panicle size than untreated plants that presented smaller panicles size
1768 ($F= 17.58$, $df= 5$, $P<0.001$). In crop season 2, the hybrid AG1090 showed the highest plant height followed by
1769 hybrids DKB590 and BRS 373 when treated with insecticide ($F= 9.82$, $df= 5$, $P<0.001$). In crop season 3, the
1770 hybrids DKB 590 and BRS 373 had the smallest panicles size when not treated with insecticide ($F= 13.24$, $df= 5$,
1771 $P<0.001$) (Figure 1).

1772 In relation to panicles weight, in crop seasons 1 ($F= 42.24$, $df= 5$, $P<0.001$) and 3 ($F= 24.26$, $df= 5$,
1773 $P<0.001$), hybrid AG 1090 exhibited greater mean values when treated with insecticide, while DKB 590 and BRS
1774 373 had the lowest panicles weight without insecticide. In crop season 3, the three hybrids treated with insecticide
1775 had higher panicles weight, while the lowest weight was obtained in hybrid BRS 373 without insecticide ($F=$
1776 13.50 , $df= 5$, $P<0.001$) (Figure 1).

1777 The infestation intensity of *D. saccharalis* in the evaluated three crop seasons was higher when the hybrids
1778 were not treated with insecticide, and consequently resulted in lower grain yields. In all treatments across evaluated
1779 crop seasons, the higher the borer infestation intensity, the lower the grain production, indicated by the significant

1780 negative correlation of Pearson's analysis, that is, the parameters were inversely proportional. Crop season 2
1781 exhibited the strongest significant correlation ($r=-0.85$), followed by crop season 3 ($r=-0.67$), and crop season 1
1782 ($r=-0.65$) (Figure 2).

1783

1784 **Discussion**

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1786 The results of the natural infestation observed in the stalks of grain sorghum hybrids demonstrated that
1787 *D. saccharalis* has a significant negative effect on grain yield. The hybrids with insecticide application had product
1788 smaller gallery size of *D. saccharalis*, higher plant height, larger panicle length and weight, and consequently
1789 higher grain yield than non treated hybrids plots. This demonstrates that chemical control is efficient when
1790 insecticide is applied at the beginning of sorghum plant development (stages V3-V4) (Magalhães and Durães
1791 2003). It shows that greater care is needed at the beginning of plant production. However, it is recommended that
1792 monitoring be continued throughout the crop cycle as these results are on specific hybrids grown in a specific
1793 production region.

1794 Although positive results in the three crop seasons for the hybrids with insecticide applications and
1795 decreasing borer infestation intensity, crop season 3 (winter season) was the one with the highest pest infestation
1796 intensity and lowest grain yield. Thus, if the number of applications were increased, the infestation intensity would
1797 decrease. Likewise, crop season 2 (summer season) was the one with the lowest infestation intensity and higher
1798 grain production, which could probably be maintained with a lower number of insecticide applications as
1799 productivity would still be high.

1800 Correlation was inversely proportional, i.e., as borer infestation intensity decreased, grain yield increased
1801 proportionally in three crop seasons. Infestation intensity is an important index and the most used parameter to
1802 assess sorghum resistance to *D. saccharalis*. The 1st crop season showed mean borer infestation rate of 12.11% in
1803 the hybrid BRS373 without insecticide treatment, which is equivalent to twice that found by Lara et al. (1997)
1804 evaluating *D. saccharalis* infestation in sorghum, who also found greater infestation intensity in the 2nd season
1805 planting, which was about 3x lower than that found in the plants without chlorantraniliprole treatment in the present
1806 study. This shows the potential of the pest for sorghum, which is considered silent due to its cryptic behavior, as
1807 the farmers usually do not monitor the borer field populations, and this insect goes unnoticed in the crops.

1808 In another study evaluating sorghum resistance to *D. saccharalis* in the same, Waquil et al. (2001) found
1809 an infestation rate of *D. saccharalis* of more than 75% in commercial sorghum hybrids, and almost 100% in

1810 sorghum lines. This shows how the infestation rate vary between sorghum genotypes, and how it is important to
1811 know these results for recommending hybrid for farmers whose crops have high *D. saccharalis* infestations. Lahiri
1812 et al. (2019) showed potential for combining host plant resistance and chemical control for *Melanaphis sacchari*
1813 (Zethner, 1897) (Hemiptera: Aphididae), and this management strategy could also be effective against *D.*
1814 *saccharalis*, meriting future evaluation.

1815 The level of control of *D. saccharalis* in sugarcane crops varies from 3-5% infestation intensity,
1816 depending on the production costs and economic revenue. Considering that grain sorghum is more tolerant to *D.*
1817 *saccharalis* attack than sugarcane, a control level of 4% infestation intensity or four weekly adults/trap (Vilela et
1818 al. 2014). However, these authors worked with sweet sorghum where the potential for yield reduction is directly
1819 in the stalk. Here, we also highlight the potential of borers in the grain. However, common sense should always
1820 be used in control decision-making; for example, even with the application of insecticide in the present study, there
1821 were moderate infestation intensity levels and still was possible to achieve high production of the than in the
1822 insecticide free condition.

1823 In a study carried out with sugarcane, sugar sorghum, and biomass sorghum, the impact of *D. saccharalis*
1824 on production parameters were evaluated and there was a negative relationship between infestation intensity and
1825 ethanol production, reducing by up to 13% in high pest infestations (Wilson et al. 2018). In a study with corn, to
1826 the authors estimated that the plants yielded an average of 2.51 g of grains for each internode bored by *D.*
1827 *saccharalis*, which is equivalent to 2.03% yield loss per plant for each internode attacked; these values can be
1828 considered a significant loss if the infestation is high (Serra and Trumper 2020).

1829 In general, the results of our study using commonly planted commercial hybrids showed sorghum
1830 susceptibility to sugarcane borer under high infestation rates, causing substantial yield losses in all hybrids when
1831 not treated with insecticide. Therefore, depending on the pest population density in the field, *D. saccharalis* can
1832 be considered one of the main pests of grain sorghum crop.

1833 According to our results, taken together we can infer that the three sorghum hybrids behaved better in
1834 first-season planting (summer season) which had the strongest significant correlation ($r = -0.85$) between borer
1835 infestation intensity and yield. In crop season 1, hybrid AG1090 showed a higher tolerance level, i.e., even without
1836 chemical control the grain yield was higher; compared to the other hybrids, it had greater plant height and produced
1837 more; and among the hybrids, it presented the lowest increase (40.54%) in grain yield when insecticide treated,
1838 followed by BRS373 with 48.94% yield increase with chemical control in crop season 1.

1839 The hybrid DKB 590 had the lowest tolerance level, whereby with insecticide treatment production
1840 increased 1.5 times in crop season 1 (149.77 %). In crop season 2, hybrid BRS 373 exhibited the lowest increase
1841 in grain yield with control (29.79%), followed by AG1090 with 33.13%, and again DKB 590 (40.79%) with greater
1842 dependence on chemical control to increase yield. Crop season 3 obtained the lowest yield among the three crops,
1843 and hybrid AG1090 responded better to chemical control with, yield increase of 105.90%, which is equivalent to
1844 more than double the yield when using chemical control, followed by DKB 590 with 54.26% which indicates more
1845 than half of the increase in production, and BRS 373 with 38.19%. Tolerant genotypes produce more than less
1846 tolerant plants, which can raise the economic injury level for insect pest, reducing the number of insecticide
1847 applications, and consequently lower selection pressure on the pest (Koch et al. 2019). In grain sorghum, so far
1848 there are no records of effective losses in relation to sugarcane borer infestation.

1849 This is the first study that effectively shows how much grain loss occurs by *D. saccharalis* attack in grain
1850 sorghum. The sugarcane borer was able to cause yield loss up to 1.5x when sorghum plants were not controlled
1851 by insecticide treatment in the less tolerant hybrid, and 50% in the more productive and tolerant hybrid. However,
1852 it is worth noting that our results show how the same hybrid in the same growing region can behave very differently
1853 in relation to tolerance and productivity, and all these factors can change the recommendation of the most suitable
1854 hybrid for a particular insect pest, region, and planting season.

1855

1856 **Declarations**

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1859 **Conflicts of interest/Competing interests:** No conflict of interest

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1863 **Consent to participate:** Not applicable

1864 **Consent for publication:** All authors participated and are in agreement with this publication.

1865 **Authors' contribution:** Camila S F. Souza performed the methodology of the essays and wrote the
1866 manuscript, Bruno H. S Souza, Priscilla T Nascimento and Simone M Mendes helped with the writing of the
1867 manuscript; Joselia C O França performed the statistical analysis of the data and graphics, Cícero B Menezes
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1873

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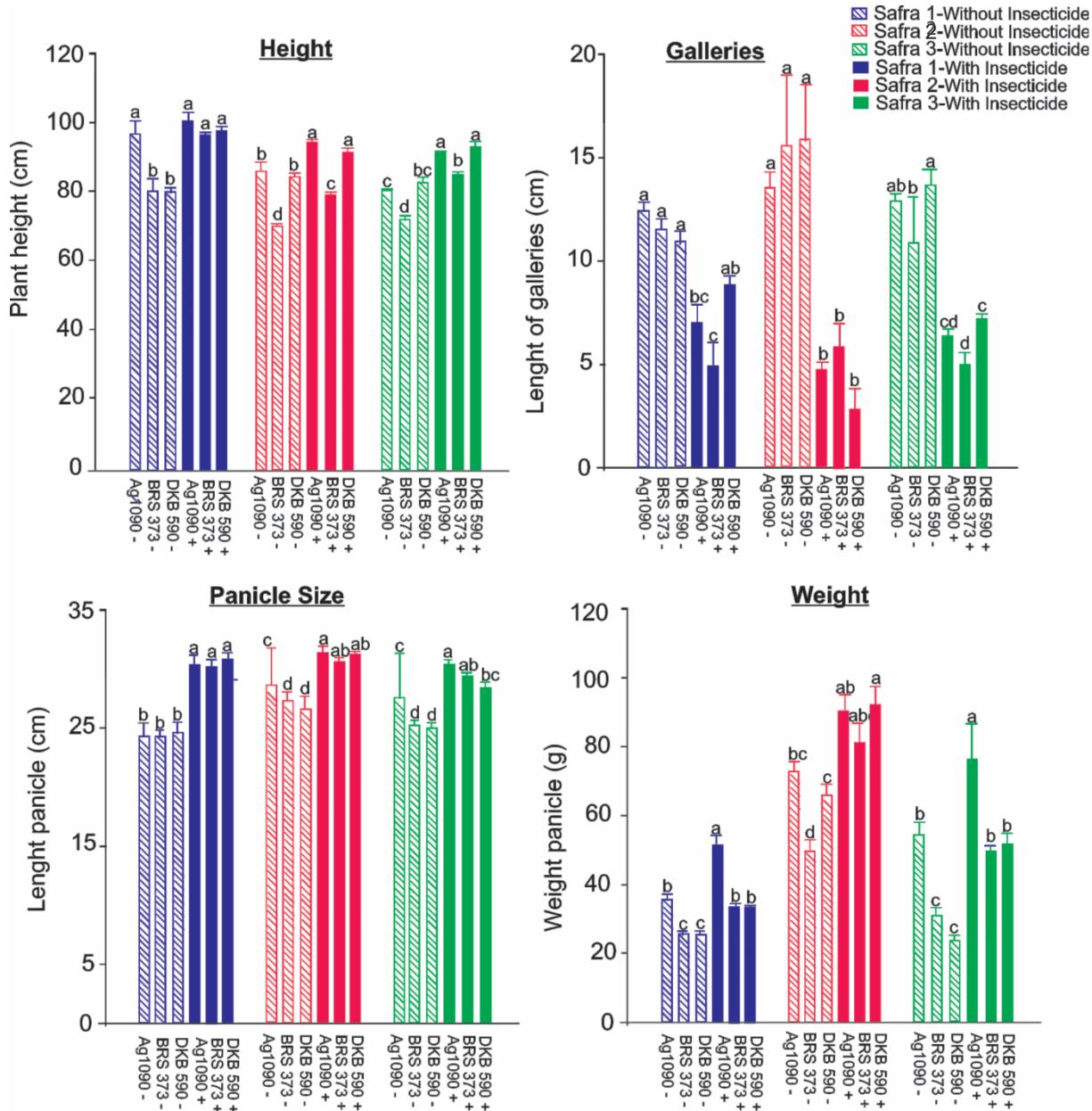
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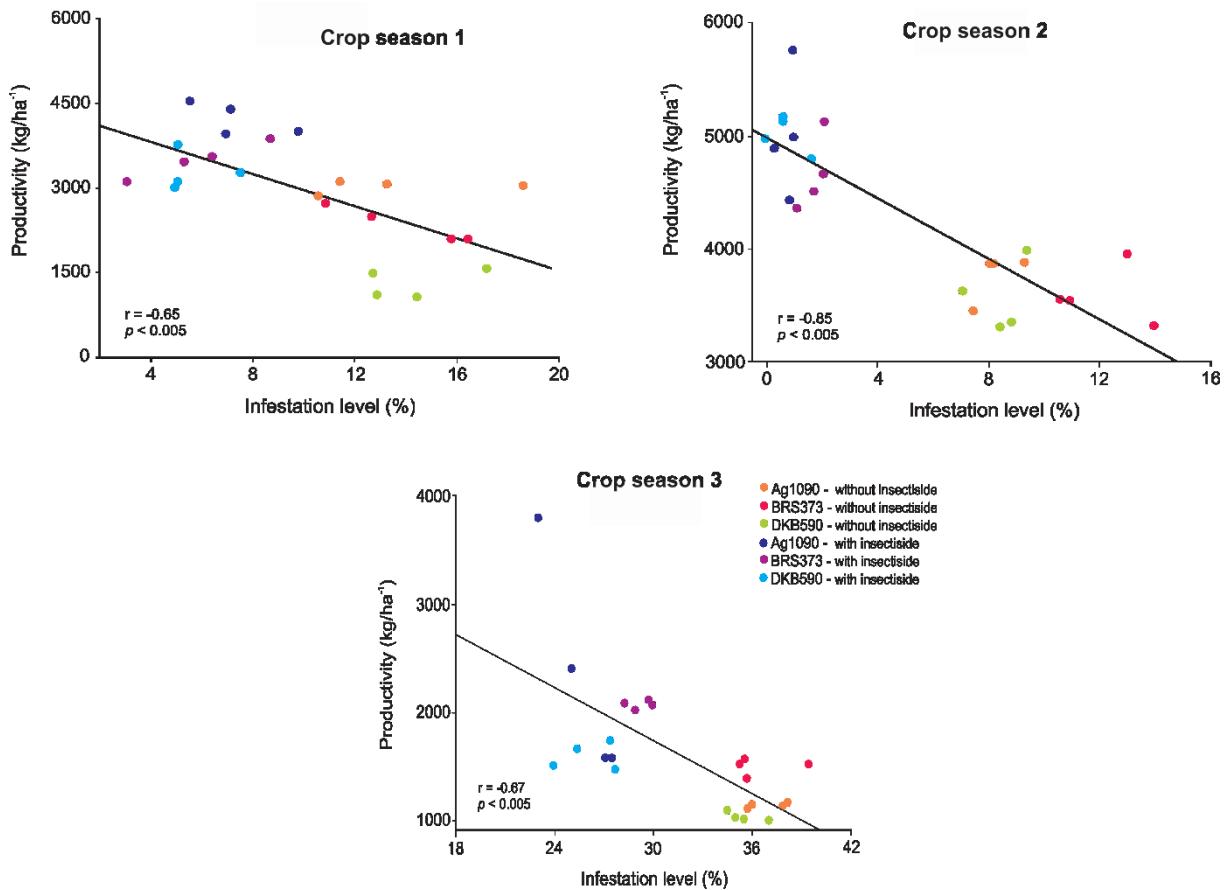
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Figure 1- Variables plant height (cm), gallery length (cm), panicle weight and length of grain sorghum hybrids treated (with plus sign (+)) and not treated (with minus sign (-)) (crosshatched) with insecticide (chlorantraniliprole) under natural infestation of *Diatraea saccharalis* in three consecutive crop seasons ($P < 0.05$).



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Figure 2. Pearson's correlation between Infestation intensity (%) and grain yield (kg ha^{-1}) of grain

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sorghum hybrids treated and not treated with insecticide under natural infestation of *Diatraea saccharalis*.

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1971 ARTICLE 3- Susceptibility of grain sorghum hybrids to major agricultural pests

1972

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1995

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2002 Ethics declarations

2003 Conflict of interest

2004 The authors declare no conflicts of interest regarding to the publication of this paper.

2005 Ethics approval

2006 Studies exempt from evaluation by the Research Ethics Committee

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2025 **Abstract**

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2027 Plant resistance is an important tactic within the precepts of Integrated Pest Management, and the existence of
2028 grain sorghum hybrids with multiple insect resistance could benefit the crop management and sustainability. The
2029 present study evaluated susceptibility of 30 grain sorghum hybrids to three major pests, *Spodoptera frugiperda*,
2030 *Diatraea saccharalis* and *Diceraeus melacanthus*. Cultivation of the hybrids and performance of experiments with
2031 each insect species were conducted separately in a greenhouse. For fall armyworm (FAW), larvae infestation took
2032 place with sorghum at the stage from four and six developed leaves and visual injury evaluation on plants were
2033 performed 7 and 14 days after infestation (DAI). For sugarcane borer (SCB), plants were infested at the same
2034 growth stage and assessed 40 DAI for the insect presence and injury. For the green-belly stink bug (GBS), the
2035 infestation was performed three days after emergence, and plants were evaluated using a damage score scale 12,
2036 19 and 26 days after infestation. Through cluster analysis, it was possible to separate the grain sorghum hybrids
2037 into groups regarding the resistance levels to each pest. The hybrid BRS373 stood out as moderately resistant to
2038 FAW; AG1090, 80G20, BRAVO, BRS373, AG1615, and IG220 were the most promising for SCB; and for GBS,
2039 the hybrids 50A40, A9735R, JADE, ENFORCER, BUSTER, 50A10 and IG244 were the most nominated. Further
2040 research should evaluate the potential chemical and morphological plant traits underlying the lower susceptibility
2041 levels found in the selected sorghum hybrids to FAW, SCB, and GBS. This information will greatly aid sorghum
2042 breeding programs focused in developing commercial hybrids possessing both insect-resistance and high-yield
2043 characteristics.

2044

2045 **Keywords:** Host plant resistance, *Sorghum bicolor*, *Spodoptera frugiperda*, *Diatraea saccharalis*, *Diceraeus*
2046 *melacanthus*.

2047 Declarations: The abstract covers the main methodology and the main results

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2055 **Introduction**

2056

2057 Sorghum, *Sorghum bicolor* (L.) Moench, is an important cereal crop used for grain, feed, and energy production.
2058 It is the fifth most produced cereal in the world, and can substitute corn in animal feed. Sorghum grains also
2059 constitute a staple food for people in some countries in Africa and Asia, and hold promise for human consumption
2060 elsewhere since they are rich in bioactive and functional compounds, and are gluten-free (Waqil et al. 2003; FAO
2061 1995; Martino et al. 2014).

2062 The demand for sorghum-based products, such as bread, pasta, cereal bars, snacks, cakes, biscuits, and
2063 beer has grown in recent years; all these products show high added value due to the benefits for human nutrition
2064 and health as an alternative to conventional cereals (Martino et al. 2014; Abdelghafar et al. 2011; Burdete et al.
2065 2010; Cardoso et al. 2014). Brazil is currently the ninth largest sorghum producer globally, being responsible for
2066 ~10% total grain sorghum production. In the 2019 crop season in Brazil, nearly 2.5 million tons of sorghum grains
2067 were produced, with the state of Goiás standing out among the Brazilian producing regions, with more than 40%
2068 of the national production, followed by Minas Gerais state (IBGE 2020). However, losses by infestation of insect
2069 pests constitute one of the major problems in sorghum production. Insect pest attacks can occur in all phenological
2070 stages of sorghum, which affect development and productivity of the plants. Fall armyworm *Spodoptera*
2071 *frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) has often been reported as one of the main pests of grain
2072 sorghum. FAW is one of the most harmful insect pests of annual crops in tropical regions of the Americas, being
2073 able to cause production losses between 17 and 39% depending on the environment and developmental stage of
2074 the attacked plants. In Brazil, FAW is also an important pest of maize, cotton, soybean, and sugarcane, among
2075 other crops of economic importance (Cruz and Turpin 1982; Boregas et al. 2013; Bernardi, et al. 2015, Goergen
2076 et al. 2016). Thus, FAW infestations in these crop plants in adjacent areas or in succession can affect damage
2077 intensity to sorghum.

2078 Another important lepidopteran pest is sugarcane borer (SCB) *Diatraea saccharalis* (Fabricius)
2079 (Lepidoptera: Crambidae). SCB is responsible for making galleries in the plants stems, weakening these structures
2080 and hampering photoassimilates translocation. In addition, indirect losses are caused by the entry of
2081 microorganisms via injured areas caused by SCB to the stem, which can lead to reduced grain production. SCB is
2082 a pest of agricultural systems not only in Brazil, but also in the USA in rice, sugarcane, and sorghum (Hayden
2083 2012; Legaspi et al. 1997). In some regions of Brazil, the damage caused by SCB can correspond to 30% of total
2084 sorghum grains production (Mendes et al. 2012, 2014).

2085 Due to cultivation of maize and sorghum in the second crop season in succession with soybeans, insect
2086 pests that were previously considered as secondary have increased in frequency of occurrence in crops grown in
2087 no-tillage systems in Brazil, as is the case with green-belly stinkbug (GBS) *Diceraeus melacanthus* (Dallas)
2088 (Hemiptera: Pentatomidae). GBS has a habit of feeding and reproducing under the straw left by preceding crops,
2089 causing significant damage to maize and sorghum plants at the beginning of vegetative development, reducing the
2090 vigor and possibly causing death of infested plants (Conab 2020; Gassen 1996; Corrêa-Ferrera; Sosa-Goméz
2091 2017). When feeding, GBS normally positions itself at the base of stem of host plants with the head positioned
2092 downward, where the stinkbug introduces the stylets to suck the phloem sap. Sap sucking is facilitated by injection
2093 of salivary enzymes that are toxic to the plant, hindering its development (Bianco 2004; Grigolli et al. 2016).

2094 In terms of genetic variability of plants in relation to insects attack, different genotypes can present higher
2095 or lower levels of resistance/susceptibility (Bastos et al. 2015). Given the fact that host plant resistance to insects
2096 is horizontal in the majority of cases, i.e. the resistance is controlled by various quantitative genes and loci, plants
2097 generally present varying response levels to pest injury, as well as in terms of effects on insect feeding, oviposition,
2098 and development. Additionally, plant resistance can be specific for a species or group of insects, while the plant
2099 remains susceptible to other pests depending on the history of insect adaptation. At the same time, multiple
2100 resistance occurs when a plant genotype presents resistance-related traits to various insect species (Kogan 1975;
2101 Lara 1991; Baldin et al. 2019), which is a desired characteristic of commercial cultivars to be used in Integrated
2102 Pest Management (IPM) systems. Ultimately, host plant resistance is a control tactic that can be applied in
2103 combination with most control tactics within the IPM precepts to minimize problems caused by excessive use of
2104 chemical insecticides.

2105 Most studies that evaluated grain sorghum genotypes for resistance to SCB and other lepidopterans in
2106 Brazil were conducted over 20 years ago, especially during the 1980s and 1990s. In these studies, sources of
2107 resistance to SCB and FAW were found to some extent through evaluation in plant parameters such as damage
2108 scores relative to larvae infestation, pest infestation intensity, and insect biology (Waqil et al. 1980; Boiça Jr. and
2109 Lara 1983; Lara et al. 1997; Waqil and Santos 1990; Waqil et al. 2001). Because these evaluations were
2110 performed long time ago, novel resistance screening phenotyping should be conducted with more recent and
2111 improved genotypes seeking the introgression of the resistant genes into germplasm of grain sorghum breeding
2112 programs. Additionally, there is no information on the responses of sorghum genotypes in terms of resistance to
2113 insect pests such as GBS that have gained recent economic importance in agricultural systems.

2114 Studying the behavioral and developmental parameters of insect pests and evaluating the injury intensity
2115 on host plants is essential to assist screening of hybrids resistant to the main grain sorghum pests and to achieve
2116 higher efficiency in the management and profitability of producers (Knolhoff and Heckel 2013). It also benefits
2117 genetic breeding programs by the production of hybrids with desirable agricultural traits and at the same time
2118 moderate-to-high resistance levels to major crop insect pests. Information available so far in the literature for
2119 potential multiple- resistance of grain sorghum genotypes to SCB, FAW, and GBS is scarce given that infestations
2120 of these pests in sorghum crops are relatively recent in the main producing regions in Brazil. Thus, this study was
2121 conducted to evaluate the susceptibility of 30 grain sorghum hybrids to three important pests of grain sorghum,
2122 fall armyworm (FAW), sugarcane borer (SCB), and green-belly stinkbug (GBS).

2123

2124 Declarations: The introduction covers the main information on the topic

2125

2126 Materials and Methods

2127

2128 Experimental conditions and grain sorghum hybrids

2129

2130 The study was carried out in a greenhouse of Embrapa milho e Sorgo, in Sete Lagoas, MG state, Brazil,
2131 under the conditions of $25 \pm 5^{\circ}\text{C}$, $70 \pm 15\%$ UR, and natural photoperiod. Thirty grain sorghum hybrids already
2132 available in the marketplace were evaluated for resistance to the three insect species (FAW, SBC, GBS), as
2133 follows: BRS373, FOX, DKB590, 1G220, AG1090, BRAVO, MSK327, 1G244, 50A10, 50A40, 50A70, 70G70,
2134 XGN1305, 80G20, MSK326, XB6022, AG1080, A9735R, AG304, 1G100, A9902, A9721R, AS1615, BUSTER,
2135 DKB550, MSK321, JADE, 1G232, ENFORCER, and MSK120.

2136 Assays was conducted in three 20-L pots used for each evaluated hybrid, with soil fertilized with 50 g of
2137 NPK 08-28-16 and 0.3% of Zn/100 kg.v. Each pot was considered a replication, totaling 90 pots used in each
2138 experiment with different insect species. Thinning of plants was performed 10 days after sowing, keeping three
2139 plants per pot. The assays with the three insect pests were carried out separately, in a completely randomized
2140 design.

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2144 **Rearing of insect species**

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2146 **Fall armyworm (FAW):** Testing insects of FAW were obtained from a rearing colony in laboratory. Briefly,
2147 larvae were reared on artificial diet based on cooked beans, wheat germ, and casein (Cruz 2000). Adults were
2148 transferred to cylindrical PVC mating cages (40 cm h x 30 cm diam.) containing moth food (10% sugar and 5%
2149 ascorbic acid in water) and white sulfite paper on the inner walls for oviposition. Collected egg masses were left
2150 to hatch in plastic bags, and neonates were transferred to the artificial diet (Cruz 2000). Newly hatched larvae
2151 obtained from the laboratory rearing colony were individually placed in 50-ml plastic cups sealed with acrylic lids
2152 (adapted from Mendes et al. 2011).

2153

2154 **Sugarcane borer (SCB):** Adults of SCB were kept in PVC cages covered with sulfite paper for oviposition. The
2155 cages were covered with voile fabric on the top that was fixed with rubber bands. Collected eggs were washed in
2156 a deionized water and 1% sodium hypochlorite solution, and then deionized water and a solution of deionized
2157 water with 1% copper sulphate to avoid microorganism contamination. Eggs were dried at room temperature, and
2158 subsequently placed in glass jars (8.5 cm x 2.5 cm) covered with voile fabric containing artificial diet based on
2159 soybean meal, sugar, and wheat germ (adapted from Hensley and Hammond 1968), where they remained until the
2160 third instar (approximately 20 days-old). Larvae were transferred to Petri dishes (5 cm diameter) with artificial
2161 diet cut into strips, due to natural larvae behavior in forming galleries. The larvae remained in Petri dishes until
2162 adult emergence. (vilela, et al 2017)

2163

2164 **Green-belly stinkbug (GBS):** Insects of GBS were reared in plastic cages (37 x 12 cm) with perforated lids to
2165 allow for air exchange, pieces of voile fabric for oviposition, water-soaked cotton wool to provide water, and a
2166 mixture of seeds of beans, sunflower, soybeans, and peanuts as food sources. The younger nymphal phases were
2167 maintained separate from the adults.

2168 Rearing of the three insect species was carried out in a climatized chamber under controlled environmental
2169 conditions (25 ± 2°C, 60 ± 10% U.R., and 12C:12E h).

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2174 **Fall armyworm assay**

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2176 For the assay with FAW, infestation with larvae was carried out when sorghum plants were at the growth
2177 stage of four completely developed leaves. Five neonate larvae (<24 hours-old) were transferred per plant using a
2178 fine paintbrush where three plants/pot were placed, totaling 15 larvae per pot. The pots were covered with voile
2179 fabric cages to prevent insects escaping from the plants.

2180 Evaluations of injury caused by FAW feeding on the plants were carried out using an damage score scale
2181 ranging from 0-9, according to that proposed by Davis in maize and adapted to sorghum (Davis and Williams
2182 1992);: 0 = no visible injury; 1 = punctures (more than one point per plant); 2 = scrapes and 1 to 3 small circular
2183 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
2184 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
2185 (>3 cm) in 1 to 2 leaves and 1 to 5 perforations or elongated lesions (<1.5 cm); 6 = 1 to 3 large elongated lesions
2186 (>3 cm) in 2 or more leaves and 1 to 3 large perforations (> 1.5 cm) on 2 or more leaves; 7 = 3 to 5 large elongated
2187 lesions (>3.5 cm) in 2 or more leaves and 1 to 3 large perforations (>1.5 cm) on 2 or more leaves; 8 = many
2188 elongated lesions (>5cm) of all sizes on the majority of the leaves; many medium to large perforations (> 5) >3
2189 cm on many leaves; 9 = many leaves affected, nearly all destroyed. Damage scores were determined at 7, 14 and
2190 21 days after FAW larvae infestation (DAI).

2191

2192 **Sugarcane borer assay**

2193

2194 When sorghum plants presented between four and six developed leaves (Magalhães et al. 2003), they
2195 were manually infested with five SCB neonate larvae per plant using a fine paintbrush. The neonate larvae (<24
2196 hours-old) were obtained from the rearing colony.

2197 Evaluation of injury by SCB was performed 40 DAI on the sorghum plants. To this end, the plants were
2198 cut close to the ground and opened longitudinally with a razor blade to detect the presence and quantify injury by
2199 SCB. Evaluated parameters were the number of healthy and bored internodes, length (cm) of the galleries made
2200 by SCB, and infestation intensity (%). The infestation intensity (I.I.) was calculated with the following formula:
2201 I.I. (%) = number of bored internodes/total number of internodes x 100 (Gallo et al. 2002).

2202

2203

2204 **Green-belly stinkbug assay**

2205

2206 For the assay with GBS, one adult stinkbug was manually infested per plant, using one plant per pot. The
2207 adults were obtained from the rearing colony and were fasted for 16 hours prior to infestation in order to stimulate
2208 feeding soon after being released onto the plant. Infestation was performed three days after seedlings emergence,
2209 and adult GBS was kept feeding on the plant for 12 days, according to the method of Gomes et al. (2011). During
2210 this period, all the pots were protected with PET perforated bottles to allow air circulation and avoid insects from
2211 escaping. Evaluations on infested plants were performed every two days to observe insect survival and replenish
2212 dead stinkbugs. The first evaluation was performed 12 DAI, soon after insects removal from plants.

2213 Evaluation of GBS injury was based on the damage score scale adopted by Roza-Gomes et al. (2011), as
2214 follows: score 0 = plants presenting no injury; 1 = leaves with punctures without reduction in plant size; 2 = slight
2215 injuries on the plant, partially rolled, with reduction in size; 3 = trapped or plant tiller; 4 = dry or dead plant (Figure
2216 3). Nineteen days after infestation, i.e. seven days after the first evaluation, the second evaluation was performed
2217 with the characterization of damage score and developmental stage of plants, according to Roza-Gomes et al.
2218 (2011). Finally, 27 DAI, i.e. 14 days after the first evaluation, a third and final injury evaluation on the plants was
2219 performed.

2220

2221 **Statistical analysis**

2222

2223 For univariate analysis the data obtained in each bioassay, for each species of insect were subjected the
2224 Shapiro-Wilk test was performed to check normality and the Bartlett test was used to check for homogeneity of
2225 variances. As data did not follow a normal distribution or exhibited heterogeneity of variances, a nonparametric
2226 test was performed using generalized linear model (GLM) and quasipoisson family, and the means of treatments
2227 were compared by Tukey's ($\alpha=0.05$). The analyses were performed using the statistical R software version 3.5.3
2228 (R Development Core Team 2014).

2229 For multivariate analysis, it was used the hierarchical clustering Unweighted Pair-Group Method using
2230 Arithmetic Averages (UPGMA) method was used in cluster dendograms fixed with three groups and using the
2231 Euclidean distance as dissimilarity unit measure. The analyses were performed using the statistical R software
2232 version 3.5.3 (R Development Core Team 2014).

2233 Declarations: The Materials and Methods explains all the methodology used and how the statistical analyzes were
2234 performed

2235

2236 **Results**

2237

2238 **Fall armyworm assay**

2239

2240 The first FAW injury evaluation on sorghum plants was carried out 7 days after infestation (DAI), where
2241 the hybrid BRS373 showed a mean injury score lower than that in the other hybrids, followed by MSK327 ($F=$
2242 2.10, $df= 29$, $P< 0.005$). These two hybrids maintained the lower injury scores at 14 DAI, together with hybrid
2243 A9735R ($F= 1.94$, $df= 29$, $P< 0.005$). The hybrid BRS373 and A9735R maintained the lower injury scores at 21
2244 DAI ($F= 2.22$, $df= 29$, $P< 0.005$). Most of the hybrids presented lower damage scores at 7 DAI in comparison with
2245 14 and 21 DAI (table 1).

2246 By using the cluster dendrogram, it was possible to separate the grain sorghum hybrids into three groups
2247 according to their similarity regarding the evaluated plant injury parameter. Hybrid BRS373 was considered
2248 moderately resistant; MSK327 and A9735R were classified as susceptible; while the other hybrids were highly
2249 susceptible to FAW (Fig. 1).

2250

2251

2252 **Sugarcane borer assay**

2253

2254 In the assay with SCB, there were significant differences ($P <0.05$) for all parameters evaluated, except
2255 infestation intensity. In the assay with SCB, the hybrids BUSTER and 50A70 had higher numbers of bored
2256 internodes ($F= 8.21$, $df= 29$, $P< 0.005$) and consequently lower numbers of healthy internodes ($F= 4.49$, $df= 29$,
2257 $P< 0.005$). In addition, no bored internodes were observed in hybrids AG1090, 80G20, AS1615, BRS373,
2258 BRAVO, and IG220, consequently did not present any injury (0 % infestation intensity) ($F= 2.12$, $df= 29$, $P<$
2259 0.005). Regarding the galleries caused SCB by feeding, hybrid 50A70 showed greater injury, followed by A6304,
2260 JADE, DKB590 and FOX ATLÂNTICA ($F= 24.37$, $df= 29$, $P< 0.005$). The hybrids AG1090, 80G20, BRAVO,
2261 BRS373, AS1615, and 1G220 did not present any galleries (Table 2).

2262 By using the cluster dendrogram, it was possible to separate the grain sorghum hybrids into three groups
2263 according to their similarity. Based on the evaluated parameters, the hybrid 50A70 was classified as highly
2264 susceptible to SCB, while the hybrids A6304, JADE, MSK321, BUSTER, FOX-ATLÂNTICA, XGN1305,
2265 DKB550 and DKB590 were considered susceptible; the other hybrids were classified as moderately resistant to
2266 SCB, highlighting AG1090, 80G20, BRAVO, BRS373, AS1615, and 1G220 by presenting no injury signals in the
2267 plants (**Fig. 2**).

2268

2269 **Green-belly stinkbug assay**

2270

2271 In the assay with GBS, GLM analysis showed no significant difference between treatments on different
2272 days of evaluations. However, we can highlight the hybrids 50A40 and A9735R presented the lowest injury scores
2273 with means of injury below one in the three evaluations at 12 DAI ($F= 2.12$, $df= 29$, $P< 0.005$), 19 DAI ($F= 1.66$,
2274 $df= 29$, $P< 0.005$) and 26 DAI ($F= 1.87$, $df= 29$, $P< 0.005$). On the other hand, the hybrids 80G20 and MSk326
2275 showed the highest scores of injury by GBS in general (Table 3).

2276 These results match the multivariate analysis it was possible to separate the grain sorghum hybrids into
2277 three groups by using the cluster dendrogram according to their similarity for the evaluated parameters. The
2278 hybrids JADE, ENFORCER, BUSTER, IG244, 50A10, 50A40, and A9735R were classified as moderately
2279 resistant, standing out the latter two hybrids that presented the lowest mean damage scores. The hybrids IG100,
2280 MSK327, AG1090, DKB550, AS1615, FOX-ATLÂNTICA, IG233, A9902, 50A70, A9721R, DKB590, and
2281 XB6022 were considered susceptible; and the other hybrids were classified as highly susceptible to GBS, among
2282 them 80G20 and MSk326 overall showed the highest damage scores of GBS injury (**Fig 3**).

2283 Declarations: All results were presented explicitly in texts, figures and tables

2284

2285 **Discussion**

2286

2287 Information on the resistance levels expressed by different grain sorghum hybrids available on the market
2288 to major insect pests infesting the crop constitute an important tool to help in selecting hybrids with multiple
2289 desirable defense traits and for use in IPM systems, especially when considering which pest species is prevalent
2290 in the given conditions under which sorghum is grown. In the present study, genetic variability between the grain
2291 sorghum hybrids in terms of the resistance levels to FAW, SCB, and GBS was investigated in greenhouse

2292 conditions. This variation in hybrid susceptibility to insect pests is important to give farmers greater support when
2293 choosing the hybrid more suitable for use in specific IPM cropping systems.

2294 In the FAW assay, evaluation of hybrids allowed the identification of BRS373, MSK327, and A9735R
2295 as presenting the lowest injury by FAW after the evaluation period. This methodology of injury evaluation was
2296 also used and allowed for the differentiation of maize and sorghum genotypes using the damage score scale for
2297 FAW, and complemented with biological evaluation of pest development. All these studies found great variation
2298 in the responses between genotypes, some of which were selected as potential resistant, whether the evaluations
2299 were carried out in the field, greenhouse or laboratory (Cortéz and Waquil 1997; Gonçalves et al. 2011; Burtet et
2300 al. 2017; Crubelati-Mulati et al. 2019).

2301 Our results indicates presence of resistance genes to FAW in the highlighted hybrids BRS373, MSK327,
2302 and A9735R, given that achieving differences between genotypes for FAW injury is not common, especially due
2303 to the larvae voracity and high polyphagy characteristics, making them able to feed on various crop plants with
2304 high degree of adaptation (Boregas 2013). Therefore, the lower damage scores found herein could be considered
2305 by sorghum producers as a reliable proxy for hybrid choice in terms of the potential for possessing some resistance
2306 level to FAW attack.

2307 Furthermore, some hybrids, although classified as highly susceptible to FAW, such as 50A40, 50A70,
2308 A9902, AS1615, MSk321, MSk326, MSK327, XGN1305, and MSK320, did not progress in the increase of injury
2309 between the two evaluations. This may be due to the resistance and/or tolerance levels of these plants. As in this
2310 study, insect parameters such as larval weight and mortality were not evaluated, but only injury to sorghum plants;
2311 minor injury may have occurred due to negative effects on insect feeding and growth (antixenosis and antibiosis
2312 resistance), as well as due to better plants ability to support and regenerate damaged tissues (tolerance). However,
2313 it is not possible to assert these questions based on our assessments, and these evaluations are suggested to be
2314 carried out in the future to characterize the presence of resistance and/or tolerance in these hybrids.

2315 In the SCB assay, the hybrids BRS373, BRAVO, AS1615, AG1090, 80G20, and IG220 highlighted
2316 without SCB-made galleries, and the hybrids MSK320, A9735R, IG233, and MSK327 as presenting both smallest-
2317 sized galleries and lowest infestation intensity. Evaluations of galleries and infestation intensity are considered the
2318 most adequate parameters for identification of resistance sources to SCB in sorghum (Waquil et al. 2001). Among
2319 those hybrids, BRAVO was also identified as one of the least susceptible to losses caused by *Sitophilus zeamais*
2320 (Motschulsky) (Coleoptera: Curculionidae) in stored grains; this information is very important given that this

2321 hybrid was also found to be less susceptible to main pests throughout the whole crop cycle until the end of the
2322 production chain, i.e. from the field up to storage (Pimentel et al. 2018).

2323 Infestation intensity is a more accurate plant parameter for predicting production losses, and this variable
2324 is also used in sugarcane and other types of sorghum as proxy of resistance levels to SCB attack (Milligan et al.
2325 2003; Araújo et al. 2019; Vilela et al. 2017). According to Gallo et al. (2002), the values shown in the parameters
2326 of infestation intensity and infestation percentage allow injury to be classified as low, with values of 0-5% and 0-
2327 25%; moderate, from 5-10% and 25-50%; regular, from 10-15% and 50-75%; elevated, from 15-25% and 75-95%;
2328 and extremely elevated, above 25% and 95%, respectively. For sugarcane, the economic threshold is fixed at 3%
2329 infestation intensity, in that an infestation intensity of 1% can lead to reductions of up to 0.28% in alcohol
2330 production, 0.49% in sugar production, and 1.5% in stems production (Arrigoni 2002). For sorghum plants, which
2331 are more tolerant to SCB attack in comparison to sugarcane, an economic threshold of 4% for infestation intensity
2332 can be used for the decision-making of pest control (Vilela et al. 2014).

2333 Studies evaluating different grain sorghum hybrids for the resistance to SCB have been performed some
2334 time ago, including the evaluation of effects of fertilization, pest biology, and plant growth parameters (Lara et al.
2335 1991; Lara et al. 1997; Cortez and Waquil 1997; López et al. 2000; Waquil et al. 2001; Bortoli et al. 2005). Results
2336 of these studies found correlations with ample genetic variability between sorghum genotypes, some of them being
2337 resistant to SCB. Of the genotypes that stood out for SCB resistance, the hybrid AF-28 is known for possessing
2338 multiple insect resistance; in addition to SCB, the hybrid presents resistance to sorghum midge *Stenodiplosis*
2339 (=*Contarinia*) *sorghicola* (Coquillett) (Diptera: Cecidomyiidae) and corn aphid *Rhopalosiphum maidis* (Fitch)
2340 (Hemiptera: Aphididae). However, hybrid AF-28 presents undesirable agronomic characteristics, such as late
2341 development and non-erect formation (Rosseto et al 1976; Boiça Junior and Lara 1983; Rosseto and Igue 1983;
2342 Lara and Perussi 1984; Lara et al. 1997). Therefore, evaluations of different genetic materials should not only take
2343 into account the resistance to the pest in question, but should also consider other plant parameters, which can result
2344 in the identification of genotypes with all desired characteristics for a cost-effective grain production.

2345 Occurrence of GBS in maize and sorghum cropping systems has been frequent in recent years due to the
2346 intensification of second crop cultivation soon after harvesting of soybean crop (Corrêa-Ferreira and Sosa-Gómez
2347 2017). This is the first study conducted for the recommendation of grain sorghum hybrids with resistance to GBS.
2348 Here, plants of sorghum hybrids were infested with one adult stinkbug, and hybrids 80G20 and MSK326 at the
2349 evaluations of 19 and 26 DAI did not present signs of recovery, and hence were the hybrids more susceptible to

2350 GBS. The hybrids 50A40 and A9735R stood out as the most resistant to GBS, which presented the lowest damage
2351 scores over the three evaluation dates.

2352 Studies carried out with maize genotypes using the injury scale for GBS are more common in the literature
2353 than for sorghum. Roza-Gomes et al. (2011) observed a mean damage score of 2.8 using the density of five
2354 stinkbugs infested on five maize plants, starting from V1 stage. In the present study, the use of the damage score
2355 scale modified from maize evaluation was an efficient method to detect differences in susceptibility to GBS among
2356 the sorghum hybrids (Roza-Gomes et al. 2011; Bridi. et al. 2016; Cruz et al. 2016; Duarte et al. 2015). Therefore,
2357 its use can be recommended in future studies with evaluation of resistance in grain sorghum to GBS.

2358 Given that GBS is usually already present in the field at beginning of maize and sorghum plants
2359 development, it is very important that the plants are able to recover from stinkbug injury along the development,
2360 otherwise they may die or show increased tillering, which is undesirable for grain sorghum production. Following
2361 stem and plant development, susceptibility to the pest is significantly reduced; however, when injury intensity is
2362 high, the chance of plant recovering over time is lower. Therefore, the hybrids responses to GBS infestation should
2363 be evaluated early on in the first stages of plant development, as was done in this study, since it is the most
2364 susceptible growth stage that the insect is able to inflict damage (Sturza et al. 2020). In this manner, in studies
2365 involving the selection of resistant grain sorghum hybrids to GBS, evaluations during these phenological stages
2366 can provide more precise and reliable results in terms of the presence of resistance and tolerance traits.

2367 The 30 grain sorghum hybrids evaluated herein showed quite different responses in relation to three
2368 major insect pests of the crop. This was expected since generally host plant resistance is specific for a determined
2369 pest species and the same genotype can be susceptible to other species. The same genotype may present moderate
2370 resistance for another pest while also showing different results depending on environmental conditions due to the
2371 genes x environment interactions. Additionally, other parameters can affect the responses of hybrids such as
2372 phenological asynchrony, plant age, prior infestation by other pests or diseases, atmospheric conditions, soil
2373 fertility, amongst others (Rossetto et al. 1976; Baldin and Bentivenha 2019).

2374 In agricultural systems where FAW historically occurs in large populations, there is a need for more
2375 frequent monitoring to support the decision making of pest control, such as chemical and biological insecticides
2376 application at adequate times when the economic threshold is reached, although this information for FAW in
2377 sorghum is still lacking. Furthermore, given that the market value of sorghum grains has been high in the last years
2378 (Conab 2018, 2020), farmers have been concerned about plant health conditions, investing more time and resources

2379 in pest control measures (Rosseto et al. 1976; Boiça Junior and Lara 1983; Rosseto and Igue 1983; Lara and Perussi
 2380 1984; Cortéz and Waquil 1997; Lara et al. 1997; Carvalho and Dias 2020).

2381 Despite the satisfactory results obtained here, where it was possible to indicate a less susceptible sorghum
 2382 hybrid for each insect pest, in light of new techniques and perspectives for research on host plant resistance,
 2383 together with the increased crop importance in terms of planted area and economic profitability for the sorghum
 2384 farmers, more research is necessary with the aim of increasing productivity and resistance levels to the major insect
 2385 pests. This is important since sorghum is a plant species that hosts the three pests throughout their phenological
 2386 development. GBS is a problem at the beginning of crop development, and is considered a limiting biotic stressor
 2387 on plant growth in sensitive stages, such that the greater the injury on the plants, the lower the chances of recovery
 2388 (Figure 4). FAW in turn can cause total leaf loss of in the attacked plant, as was observed in this study in hybrids
 2389 with high damage scores (8-9), totally undermining plants photosynthesis. SCB that feed on the stem, makes
 2390 galleries that weaken the plants, rotting by favoring the entry of microorganisms leading to mycotoxins
 2391 accumulation, death of the apical meristem, reduction of sap flow, and general plant debilitation (Mendes et al.
 2392 2014; Silva et al. 2014) (Figure 4).

2393 As the main conclusions of this study in terms of the lower levels of susceptibility to attack by the three
 2394 major insect pests, for improved management of FAW the hybrids BRS373, MSK327, and A9735R are
 2395 recommended; for SCB, the highlighted hybrids are AG1090, 80G20, BRAVO, BRS373, AG1615, and IG220;
 2396 and for GBS, the hybrids 50A40, A9735R, JADE, ENFORCER, BUSTER, 50A10, and IG244 are the most
 2397 promising. Further research should evaluate the potential chemical and morphological plant traits underlying the
 2398 lower susceptibility levels found in the selected sorghum hybrids to FAW, SCB, and GBS. This information will
 2399 greatly aid sorghum breeding programs focused in developing commercial hybrids possessing both insect-
 2400 resistance and high-yield characteristics.

2401 Declarations: All results were discussed and based on the literature and original explanatory figures were added.
 2402 All authors contributed to the realization of this manuscript.

2403

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2677 Table 1. Mean (\pm SE) injury scores (1 to 9) caused by *Spodoptera frugiperda* larvae on grain sorghum hybrids at
 2678 7, 14 and 21 days after infestation (DAI).

HÍBRIDOS	7 días	14 días	21 días
1G100	7,11 \pm 0,11	8,56 \pm 0,44	9,00 \pm 0,00
1G220	7,11 \pm 0,11	8,89 \pm 0,11	9,00 \pm 0,00
1G233	6,44 \pm 0,29	8,22 \pm 0,22	8,78 \pm 0,11
1G244	7,89 \pm 0,29	9,00 \pm 0,00	9,00 \pm 0,00
50A10	7,89 \pm 0,40	9,00 \pm 0,00	9,00 \pm 0,00
50A40	8,11 \pm 0,11	8,56 \pm 0,44	8,89 \pm 0,11
50A70	7,11 \pm 0,68	8,33 \pm 0,67	8,44 \pm 0,56
70G70	7,44 \pm 0,29	8,67 \pm 0,19	9,00 \pm 0,00
80G20	7,11 \pm 0,40	8,56 \pm 0,22	9,00 \pm 0,00
A6304	7,00 \pm 0,19	9,00 \pm 0,00	9,00 \pm 0,00
A9721R	6,89 \pm 0,11	8,22 \pm 0,22	9,00 \pm 0,00
A9735R	6,33 \pm 0,69	6,78 \pm 1,90 **	7,00 \pm 2,00 **
A9902	7,89 \pm 0,48	9,00 \pm 0,00	9,00 \pm 0,00
AG1080	7,33 \pm 0,19	8,22 \pm 0,11	9,00 \pm 0,00
AG1090	6,78 \pm 0,22	8,44 \pm 0,29	8,89 \pm 0,11
AS1615	7,67 \pm 0,38	8,44 \pm 0,29	9,00 \pm 0,00
BRAVO	7,11 \pm 0,11	8,44 \pm 0,29	8,89 \pm 0,11
BRS373	3,11 \pm 1,57 ***	3,89 \pm 1,95 ***	5,44 \pm 2,00 ***
BUSTER	8,22 \pm 0,11	9,00 \pm 0,00	9,00 \pm 0,00
DKB550	7,89 \pm 0,44	8,78 \pm 0,22	9,00 \pm 0,00
DKB590	7,22 \pm 0,22	8,78 \pm 0,22	8,89 \pm 0,11
ENFORCER	7,56 \pm 0,40	8,78 \pm 0,22	9,00 \pm 0,00
JADE	7,78 \pm 0,40	9,00 \pm 0,00	9,00 \pm 0,00
MSK321	6,89 \pm 0,48	8,00 \pm 0,33	8,78 \pm 0,22
MSK326	7,11 \pm 0,80	8,89 \pm 0,11	9,00 \pm 0,00
MSK327	5,33 \pm 1,95 **	6,67 \pm 1,84 **	7,67 \pm 1,33
XB6022	7,67 \pm 0,19	9,00 \pm 0,00	9,00 \pm 0,00
XGN1305	7,22 \pm 0,78	8,67 \pm 0,33	9,00 \pm 0,00
MSK320	7,56 \pm 0,56	8,44 \pm 0,56	8,78 \pm 0,22
FOX ATLÂNTICA	7,11 \pm 0,40	8,89 \pm 0,11	9,00 \pm 0,00

2679 Asterisks refer to significance. Means followed by *** = highly significant, ** significant and no asterisk = low
 2680 significance, ns= not significant by the Tukey test ($P < 0.05$).
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2686 Table 2. Means (\pm SE) of numbers healthy and bored internodes, gallery length (cm) and caused by *Diatraea*
 2687 *saccharalis* in grain sorghum hybrids. I.I = infestation intensity.

Hybrids	Healthy internodes	Bored internodes	Galleries (cm)	I.I (n.s)
1G100	4,11 \pm 0,44	0,78 \pm 0,29	2,89 \pm 0,39	13,36 \pm 3,72
1G220	5,44 \pm 0,78 **	0,00 \pm 0,00	0,00 \pm 0,00	0,00 \pm 0,00
1G233	4,33 \pm 0,67	0,22 \pm 0,11 **	4,33 \pm 2,19	7,14 \pm 4,96
1G244	3,89 \pm 0,59	0,44 \pm 0,11	4,50 \pm 1,26	11,11 \pm 4,24
50A10	3,56 \pm 0,40	0,56 \pm 0,11	4,00 \pm 0,29	13,33 \pm 3,26
50A40	3,11 \pm 0,40	0,78 \pm 0,11	2,56 \pm 0,06	18,52 \pm 1,85
50A70	1,00 \pm 0,69 ***	1,56 \pm 0,29	12,83 \pm 0,05 ***	64,81 \pm 20,87
70G70	3,56 \pm 0,11	0,44 \pm 0,11	4,67 \pm 0,88	13,33 \pm 2,55
80G20	5,11 \pm 0,80	0,00 \pm 0,00	0,00 \pm 0,00	0,00 \pm 0,00
A6304	1,89 \pm 0,29 ***	1,00 \pm 0,19	11,08 \pm 0,74 **	33,33 \pm 6,42
A9721R	6,67 \pm 0,58 ***	1,00 \pm 0,33	4,14 \pm 0,18	12,59 \pm 2,63
A9735R	4,67 \pm 0,58	0,33 \pm 0,00 **	3,67 \pm 0,33	5,29 \pm 0,26
A9902	5,56 \pm 0,29 **	1,00 \pm 0,33	2,89 \pm 0,11	14,52 \pm 4,73
AG1080	3,89 \pm 0,29	0,67 \pm 0,00	4,08 \pm 0,36	15,00 \pm 0,00
AG1090	4,00 \pm 0,51	0,00 \pm 0,00	0,00 \pm 0,00	0,00 \pm 0,00
AS1615	5,33 \pm 1,53 **	0,00 \pm 0,00	0,00 \pm 0,00	0,00 \pm 0,00
BRAVO	4,89 \pm 0,40	0,00 \pm 0,00	0,00 \pm 0,00	0,00 \pm 0,00
BRS373	3,44 \pm 0,44	0,00 \pm 0,00	0,00 \pm 0,00	0,00 \pm 0,00
BUSTER	4,00 \pm 0,58	1,78 \pm 0,56 ***	4,74 \pm 0,38	29,59 \pm 8,56
DKB550	2,33 \pm 0,19	0,78 \pm 0,11	3,61 \pm 0,45	24,07 \pm 4,90
DKB590	4,00 \pm 0,84	0,89 \pm 0,40	5,86 \pm 0,58 **	22,41 \pm 10,20
ENFORCER	5,78 \pm 0,87 **	0,89 \pm 0,40	3,42 \pm 0,79	12,79 \pm 6,75
JADE	2,89 \pm 0,22	0,67 \pm 0,00	8,83 \pm 0,93 **	19,44 \pm 1,60
MSK321	2,33 \pm 0,38	1,22 \pm 0,40	3,25 \pm 0,43	37,96 \pm 14,55
MSK326	2,89 \pm 0,11	0,44 \pm 0,11	4,67 \pm 0,67	14,81 \pm 3,34
MSK327	4,00 \pm 0,38	0,33 \pm 0,00 **	2,50 \pm 0,14	7,22 \pm 0,56
XB6022	5,11 \pm 0,95	0,89 \pm 0,22	4,44 \pm 0,29	16,22 \pm 4,65
XGN1305	3,56 \pm 1,09	0,89 \pm 0,40	3,33 \pm 1,76	23,61 \pm 13,68
MSK320	5,56 \pm 0,68 **	0,22 \pm 0,11 **	7,08 \pm 0,74	4,07 \pm 2,06
FOX ATLÂNTICA	2,89 \pm 0,29	1,22 \pm 0,29	5,64 \pm 0,07 **	28,52 \pm 6,46

2688 Asterisks refer to significance. Means followed by *** = highly significant, ** significant and no asterisk = low
 2689 significance, ns= not significant by the Tukey test ($P < 0,05$).

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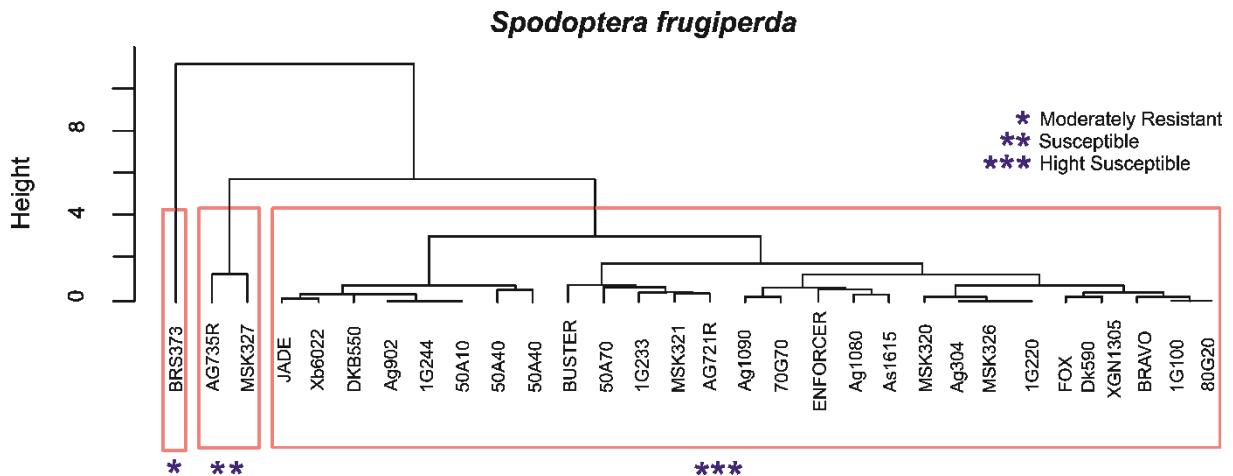
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2697 Table 3. Mean (\pm SE) injury scores (1 to 4) of *Diceraeus melacanthus* in grain sorghum hybrids at 12, 19, and 26
 2698 days after infestation (DAI) by adult stinkbug.

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

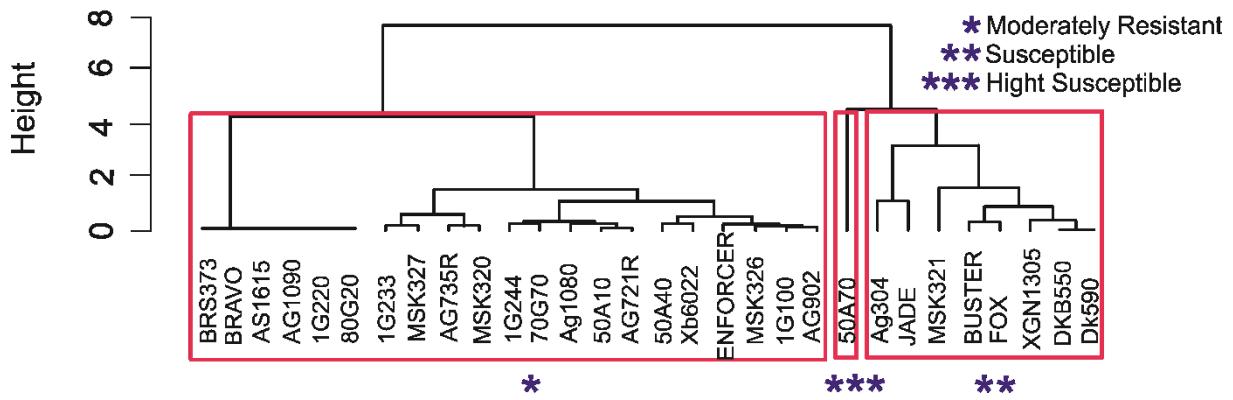
2699 ns= not significant by the Tukey test ($P < 0.05$).
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2702 **Fig 1** Dendrogram of the cluster analysis based on the Euclidean distance and grouping by means of UPGMA, in
 2703 terms of damage scores by *Spodoptera frugiperda* larvae on grain sorghum hybrids at 7 and 14 days after
 2704 infestation

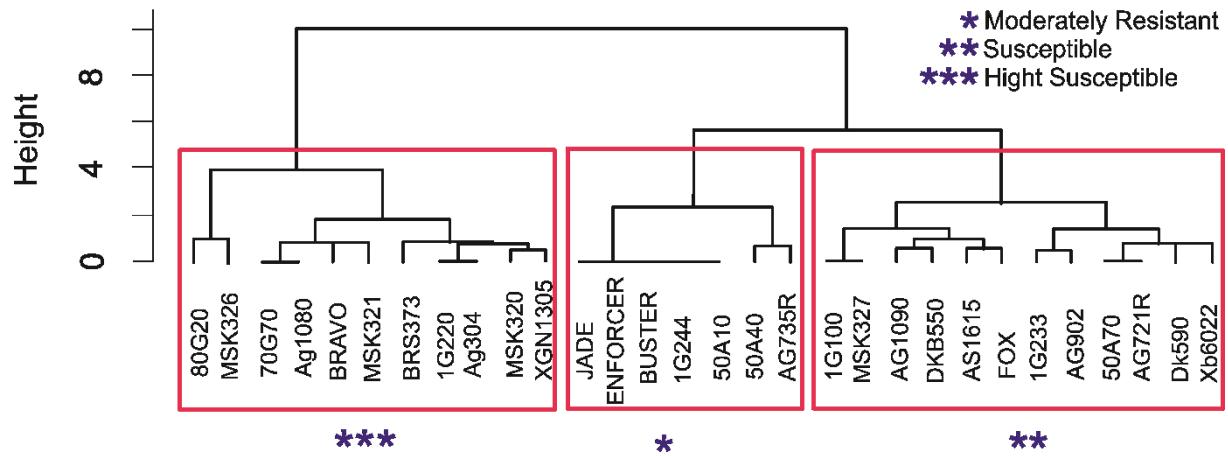
Diatraea Sacharalis



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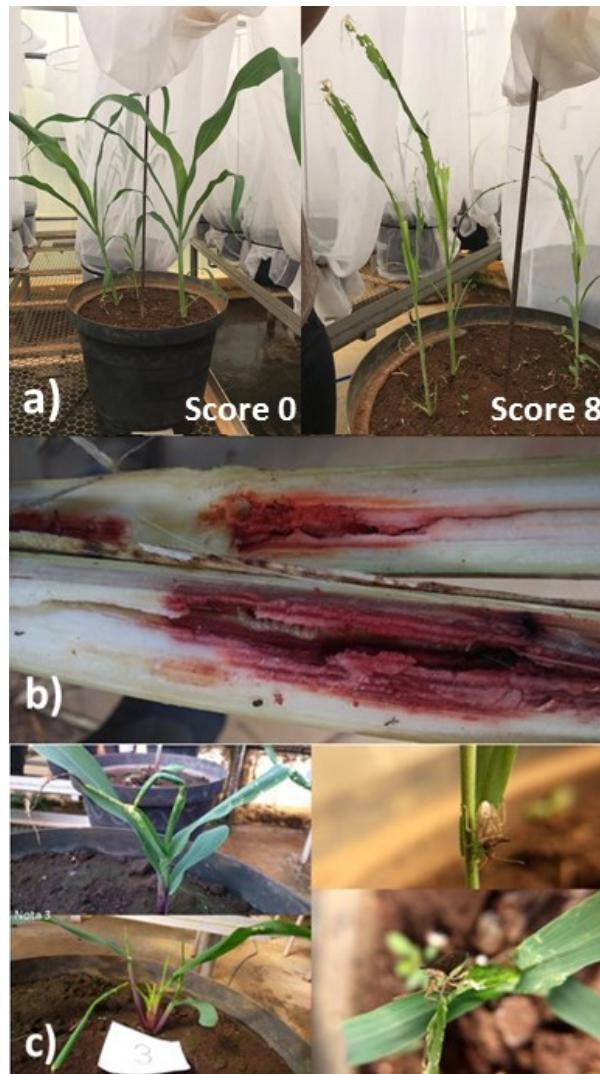
2706 **Fig. 2** Dendrogram of the cluster analysis based on the Euclidean distance and grouping by means of UPGMA, in
 2707 terms of numbers healthy and bored internodes, gallery length and infestation intensity by *Diatraea saccharalis* in
 2708 grain sorghum hybrids

Diceraeus melacanthus



2709 **Fig. 3** Dendrogram of the cluster analysis based on the Euclidean distance and grouping by means of UPGMA,
 2710 in terms of damage scores of *Diceraeus melacanthus* in grain sorghum hybrids at 12, 19, and 26 days after infestation
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2715 Figure 4. Scores on sorghum plants caused by *S. frugiperda* (a); *D. saccharalis* (b); and *D. melacanthus* (c)



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2717 Figure 5- Green-belly stinkbug injury based on the damage score scale adapted by Roza-Gomes et al. (2011) (0-
2718 4) to maize injury