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Field damage of sorghum (*Sorghum bicolor*) with reduced lignin levels by naturally occurring insect pests and pathogens

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Abstract Sorghum (*Sorghum bicolor* (L.) Moench) *brown midrib* (*bmr*) mutant lines have reduced levels of lignin, which is a potentially useful trait for bioenergy production, but the effects of this trait on insect and plant pathogen interactions are unknown under field conditions. Field-grown *bmr6*, *bmr12*, and wild-type (WT) plants were examined for insect and disease damage. In most cases, observed frequency, population, or leaf area damage caused by insects or pathogens on *bmr6* or *bmr12* plants were not greater than those observed on WT plants in the field or laboratory assays. European corn borers [*Ostrinia nubilalis* (Hübner)(Lepidoptera: Pyralidae)] often caused lower amounts of leaf damage to *bmr6* leaves compared to *bmr12* and sometimes WT leaves in the field study. Leaf damage by corn earworms [*Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)] and fall armyworms [*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)] in laboratory assays was often lower for *bmr* versus WT leaves. Incidence of disease lesions was significantly higher on *bmr6* compared to WT plants for one of three samplings in 2011, but the opposite trend was observed overall in 2012 and no significant differences were noted in 2013. When corn earworms and fall

armyworms were fed the excised pith, *bmr6* and/or *bmr12* pith caused significant mortality to one or both insect species in all 3 years. Damage variability between the 3 years may have been due to hotter and drier than normal conditions in 2012. Thus, *bmr* lines of sorghum suitable for bioenergy production have potential for sustainable production in the field.

Keywords Anthracnose · Bioenergy · Disease resistance · *Helicoverpa zea* · Insect resistance · *Sorghum bicolor* · *Spodoptera frugiperda*

Key message

- Lignin can be an important contributor to insect and pathogen resistance for sorghum. Our laboratory studies have indicated few negative effects on low-lignin sorghum lines for insects or pathogens tested. We hypothesized that this trend would hold under natural field conditions.
- No consistent increase in insect or pathogen damage for the low-lignin lines was observed.
- There were several cases where insect resistance was higher in low-lignin lines.
- Low-lignin sorghum lines tested look promising for sustainable production.

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Introduction

Bioenergy production using biomass is a potential means to satisfy the need for liquid transport fuels in a sustainable manner (United States Department of Energy 2011). The

presence of lignin within plant cell walls is one impediment to the saccharification and fermentation of biomass into ethanol (Dien et al. 2009). Reduction of lignin levels in bioenergy feedstocks through breeding, mutational genetics, or genetic engineering may interfere with pest resistance, because the presence of lignin in cell walls or induced lignin biosynthesis has been reported as part of defense responses to biotic stresses (Swain 1979; Vance et al. 1980; Santiago et al. 2013; Zhao and Dixon 2014). Reduced expression of different lignin biosynthetic enzymes has led to increased pathogen susceptibility in wheat (Bhuiyan et al. 2009a, b).

Two well-characterized brown midrib mutants of sorghum [*Sorghum bicolor* (L.) Moench] are *bmr6* and *bmr12* (Bout and Vermeris 2003; Saballos et al. 2009; Sattler et al. 2009). *bmr6* is due to a nonsense mutation that disrupts the cinnamyl alcohol dehydrogenase (CAD) gene (Saballos et al. 2009; Sattler et al. 2009). *bmr6* plants have significantly lower levels of lignin than wild type (WT) and incorporate cinnamyl aldehyde into lignin (Palmer et al. 2008; Sattler et al. 2009). *bmr12* is due to a nonsense mutation that disrupts a caffeic acid *O*-methyl transferase (COMT) gene, and *bmr12* plants contain lower levels of lignin with a reduction in sinapyl subunits as compared to WT (Bout and Vermeris 2003). These lines have reduced lignin with altered composition and are easier to saccharify (Dien et al. 2009). It has also been shown that *bmr6* and *bmr12* lines have enhanced resistance to *Fusarium* pathogens (Funnell-Harris et al. 2010). However, insect pest resistance has not been examined in the field, which if compromised would prevent adoption of *bmr6* or *bmr12* for bioenergy or other uses. On the other hand, changes to the lignin biosynthetic pathway results in the accumulation of several phenolic metabolites (Palmer et al. 2008), which may increase insect resistance of *bmr* plants compared to WT (Dowd and Sattler 2015). Many of these compounds are also associated with disease resistance (Funnell-Harris et al. 2014).

The chewing insects, corn earworm [*Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)] and fall armyworm [*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)], can be serious pests of sorghum, maize, and other crops and wild grasses (Metcalf and Metcalf 1993; Huang et al. 2013). Because laboratory studies with representative insects (Dowd and Sattler 2015) and pathogens (Funnell-Harris et al. 2014) have generally indicated no enhanced susceptibility of the *bmr6* and *bmr12* sorghum compared to WT, we hypothesized that similar results would occur when the plants were exposed to a potentially wide spectrum of naturally occurring insects and pathogens in the field. Here, we examine differences in resistance between near-isogenic wild-type and *bmr* sorghum to endemic pests in field settings and expose field-grown plant tissue to corn

earworms and fall armyworms under controlled laboratory conditions.

Materials and methods

Insects used in assays

The *H. zea* and *S. frugiperda* were reared on a pinto bean-based diet at 27 ± 1 °C, 50 ± 10 % r.h., and a 14:10 light:dark photoperiod, as described previously (Dowd 1988). First instar caterpillars were used in all assays.

Plants

Near-isogenic BTx623 *bmr6* and *bmr12* sorghum lines, and WT, (Pedersen et al. 2006) were used. Plants were grown adjacent to each other in a field plot located in Peoria, IL, as described for maize (Dowd and White 2002), and locations of plants in the field site were randomized each year. Seeds were planted in Redi Earth (Scotts Sierra, Milpitas, CA) in flats in the greenhouse, and 25 plants of each type were transplanted outside after three leaves were present. Seedlings were fertilized with time-release fertilizer when transplanted, and then fertilized weekly with foliar applications after plants reached 30 cm in height (Dowd and White 2002). Rows were 60 cm apart, and plants in rows were 30 cm apart. Natural rainfall was supplemented with irrigation in order to produce averages of 2.5 cm of rainfall per week.

Field damage ratings

Each field-grown plant was rated for naturally occurring chewing and sucking insect incidence. Every plant was inspected weekly, and ratings commenced or resumed once new insect or disease damage was noted. Chewing insect damage (Metcalf and Metcalf 1993) was rated by determining the total area feeding equivalent damage (mm^2) as determined by eye with a ruler. The severity of aphid infestation was rated on a 0–4 scale, with 0 = no aphids present, 1 = aphids present but less than 20, 2 = 20–100 aphids present, 3 = more than 100 aphids present but remaining on one leaf, and 4 = more than 100 aphids present and multiple leaves colonized. We evaluated disease by considering the presence of lesions only, under naturally occurring conditions. The area often has high humidity in the summer, and we used overhead irrigation to supplement naturally occurring rainfall when rain was less than 2.5 cm per week on average. During the first year of the study, anthracnose was found to be the identifiable cause of the lesions (see “Results” section), and these appeared to be the only type of lesions seen throughout the 3 years of the study. Incidence per plant and total area of

lesions were determined in all years by adding numbers of equivalent cm^2 or mm^2 to the nearest measure of each lesion (depending on general lesion size) as determined by eye with a ruler, and sizes of individual lesions were also rated in 2012 and 2013. New damage occurred at such a frequency that leaves were only scored once, and only the top five leaves were rated at each sample date as that was where new damage occurred.

Laboratory bioassays

Leaf section bioassays were performed using Petri dishes with tight fitting lids as described by Dowd et al. (2007). Leaves (2nd from top) on mature plants that had formed flower heads were removed for laboratory assays. Leaves from at least ten plants of each type that were as uniform as possible were used from plants that were in an equivalent growth stage as indicated by developmental stage of the flower (seed formed but still green). Ten first instar larvae were added to each dish. Feeding rates on leaves were evaluated by determining 1 mm^2 hole equivalents as described previously by Dowd et al. (2007). Survivors were weighed using a Mettler AE104 analytical balance, which is accurate to 0.01 mg (Dowd and Sattler 2015).

Stem sections were removed from stalks of plants, at the soft dough stage of grain fill prior to black layer deposition. The stalk section between the top (flag) leaf and the leaf immediately below that was removed for assays because the color of *bmr* pith was similar to that of the *bmr* midribs. Pith was removed from the stalks using cork borers, and pith sections approximately 2 cm long were placed in Petri dishes as described for leaf section bioassays. Ten first instar larvae were added to each dish. Insect mortality was recorded for pith feeding assays, but insect feeding was not rated, because it was not possible to quantitatively determine the amounts of feeding within and on the surface of pith sections because of uneven surfaces (Dowd and Sattler 2015).

Statistical analysis

Mortality and incidences of pests in different treatments were compared by χ^2 analysis using SAS Proc Freq. Feeding rates, survivor weights, and aphid population ratings were compared by analysis of variance using SAS Proc GLM. Windows Version 8.0 of the SAS software was used.

Results

Insect damage on field-grown plants

During 2011 through 2013, there were no differences in the incidence of chewing insect damage for any of the sorghum

lines, except for a significantly ($\chi^2 = 3.90$, $P = 0.048$) lower incidence of damage of *bmr6* compared to *bmr12* leaves for the first sampling in 2011 (July 22) (Table 1), and significantly lower percentage of *bmr6* leaves compared to WT or *bmr12* leaves with more than 20 mm^2 of damage in 2012 (July 24) (20.8, 56.0 and 66.7 %, respectively). The amount of damage was generally not significantly higher on any of the *bmr* leaves compared to WT. Chewing insect damage was significantly lower on *bmr6* compared to WT ($F = 6.42$, $P = 0.030$) and *bmr12* ($F = 5.27$, $P = 0.039$) lines on the third sample date (August 1) in 2013. On the first sample date in 2012 (July 24), damage to *bmr12* leaves was significantly higher than for *bmr6* leaves ($F = 10.95$, $P = 0.0019$). Chewing insect damage overall was much lower in 2012 and 2013 than 2011. Based on the type of feeding damage and insects observed, the damage was primarily due to Japanese beetles [*Popillia japonica* (Fitch) (Coleoptera: Scarabaeidae)] in 2011. In contrast, primarily caterpillar damage occurred in 2012 and 2013 due to European corn borers [*Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae)]. A limited amount of boring damage was observed in stalks examined in 2012.

The incidence of aphids [primarily *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae)] (Fig. 1a) was not significantly different for any of the sorghum lines in 2011. In the second sampling date in 2012 (August 6), *bmr6* plants had a significantly ($F = 7.26$, $P = 0.0070$) lower incidence than the WT (Table 1). Aphid incidence was significantly lower on *bmr6* and *bmr12* compared to WT plants on the last two sample dates of August 13 ($F = 7.79$, $P = 0.0053$; $F = 4.47$, $P = 0.034$, respectively) and August 26 ($F = 21.54$, $P < 0.0001$; $F = 16.83$, $P < 0.0001$, respectively) in 2013. On the third sampling date of 2012 (August 22), the density rating of aphids per plant was significantly ($F = 5.29$, $P = 0.044$) higher on *bmr12* plants compared to *bmr6* plants.

Disease damage on field-grown plants

The incidences of foliar disease were similar for all three sorghum lines examined over the three years, although the incidence was significantly higher on *bmr6* plants, compared to WT ($\chi^2 = 6.46$, $P = 0.011$), for the first sample in 2011 (July 22), while the disease incidence on this line was lower than on *bmr12* ($\chi^2 = 4.36$, $P = 0.037$) or WT ($\chi^2 = 5.35$, $P = 0.021$) plants for the first sample of 2012 (July 24) (Table 2). The size (2011) or the number (2012) of lesions was generally not significantly different between the lines, although the mean size of lesion damage for the second sample date in 2011 (September 23) and fourth sample date in 2013 (August 14) was significantly less for *bmr6* plants compared to *bmr12* ($F = 26.77$, $P < 0.0001$;

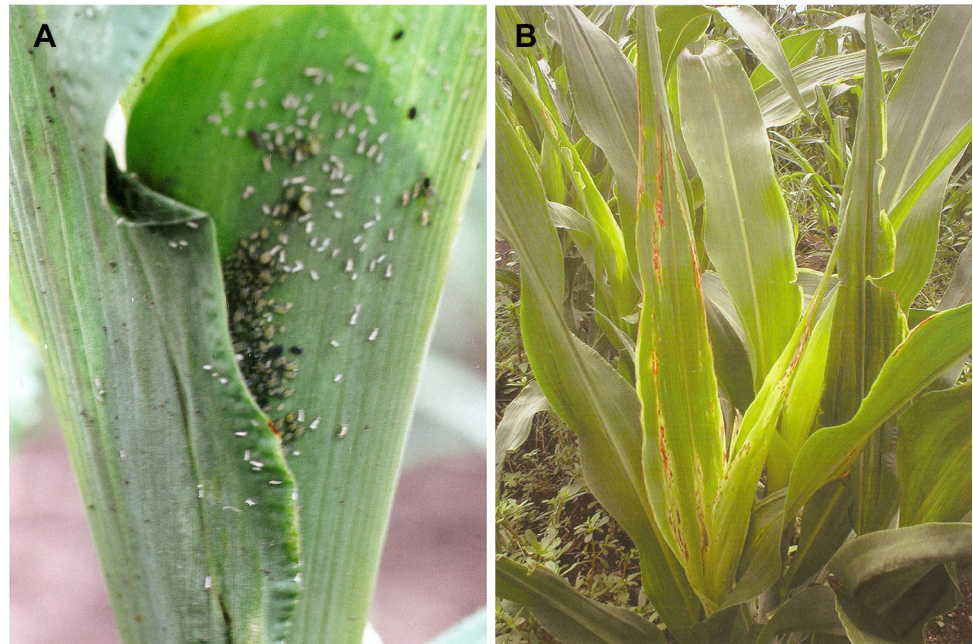
Table 1 Insect damage on leaves of field-grown sorghum plants

Sample date, line	Chewing % incidence	Chewing damage (cm ²)	Aphid % incidence	Aphid population
2011				
July 22				
Wild type	47.1ab	15.4 ± 2.8a	0.0a	0.0 ± 0.0
<i>bmr6</i>	20.8b	14.4 ± 2.6a	0.0a	0.0 ± 0.0
<i>bmr12</i>	46.7a	12.5 ± 1.8a	7.1a	2.0 ± 0.6
September 23				
Wild type	31.2a	0.9 ± 0.3a	0.0a	0.0 ± 0.0
<i>bmr6</i>	18.2a	0.2 ± 0.1a	0.0a	0.0 ± 0.0
<i>bmr12</i>	23.1a	0.4 ± 0.1a	0.0a	0.0 ± 0.0
October 22				
Wild type	0.0	0.0 ± 0.0	25.0a	1.0 ± 0.0
<i>bmr6</i>	0.0	0.0 ± 0.0	13.6a	1.0 ± 0.0
<i>bmr12</i>	0.0	0.0 ± 0.0	38.5a	1.0 ± 0.0
2012				
July 24				
Wild type	92.0a	0.29 ± 0.06ab	4.0a	1.0 ± 0.0
<i>bmr6</i>	87.5a	0.17 ± 0.04a	4.2a	1.0 ± 0.0
<i>bmr12</i>	95.8a	0.40 ± 0.06b	0.0a	0.0 ± 0.0
August 6				
Wild type	0.0a	0.0 ± 0.0	75.0a	1.8 ± 0.2a
<i>bmr6</i>	4.2a	6.0 ± 0.0	37.5b	1.9 ± 0.4a
<i>bmr13</i>	3.8a	3.0 ± 0.0	50.0ab	1.6 ± 0.2a
August 22				
Wild type	0.0	0.0 ± 0.0	37.5a	1.7 ± 0.4ab
<i>bmr6</i>	0.0	0.0 ± 0.0	24.0a	1.2 ± 0.2a
<i>bmr12</i>	0.0	0.0 ± 0.0	24.0a	2.2 ± 0.4b
2013				
July 5				
Wild type	7.7a	8.0 ± 0.0	0.0	0.0 ± 0.0
<i>bmr6</i>	4.2a	7.0 ± 0.0	0.0	0.0 ± 0.0
<i>bmr12</i>	8.3a	11.5 ± 6.4	0.0	0.0 ± 0.0
July 19				
Wild type	25.0a	7.2 ± 1.3a	0.0	0.0 ± 0.0
<i>bmr6</i>	18.2a	6.5 ± 2.8a	0.0	0.0 ± 0.0
<i>bmr12</i>	26.3a	22.6 ± 4.6a	0.0	0.0 ± 0.0
August 1				
Wild type	31.2a	23.8 ± 3.1a	0.0	0.0 ± 0.0
<i>bmr6</i>	38.5a	8.7 ± 1.1b	0.0	0.0 ± 0.0
<i>bmr12</i>	33.3a	22.9 ± 3.8a	0.0	0.0 ± 0.0
August 13				
Wild type	0.0	0.0 ± 0.0	56.2a	1.7 ± 0.3a
<i>bmr6</i>	0.0	0.0 ± 0.0	13.6b	1.7 ± 0.1a
<i>bmr12</i>	4.5	28.0 ± 0.0	22.7b	1.6 ± 0.2a
August 26				
Wild type	0.0	0.0 ± 0.0	72.7a	1.4 ± 0.2a
<i>bmr6</i>	0.0	0.0 ± 0.0	10.0b	1.7 ± 0.2a
<i>bmr12</i>	0.0	0.0 ± 0.0	10.0b	2.0 ± 0.3a

Chewing insect damage values and sucking insect population values are means ± standard errors. Sucking insect populations are based on a 0 (minimum) to 4 (maximum) infestation level. The values of like studies followed by different letters are significantly different at $P < 0.05$ by χ^2 analysis (percent incidences) or analysis of variance (feeding damage, aphid population). October 22 ratings in 2011 were on tillers and August 26 ratings in 2013 included tillers

Fig. 1 Representative insect and disease seen on WT sorghum in 2011.

a Representative example of corn leaf aphids [*Rhopalosiphum maidis* (Fitch)] seen on WT sorghum on October 22, 2011.
b Representative example of anthracnose (*Colletotrichum sublineola* Henn. Ex. Sacc. and Trotter 2013) seen on WT sorghum on September 23, 2011



$F = 16.88$, $P < 0.0001$, respectively) or WT ($F = 16.86$, $P = 0.0002$; $F = 8.55$, $P = 0.0054$). The size of lesions was significantly larger for the *bmr12* line compared to *bmr6* or WT for the third sample date in 2012 (August 22) ($F = 7.36$, $P = 0.015$; $F = 7.03$, $P = 0.017$, respectively), and third (August 1)(WT, $F = 8.14$, $P = 0.0010$) and fourth (August 14) (*bmr6*, $F = 5.18$, $P = 0.033$) sample dates in 2013 (Table 2). Based on comparisons of lesion type (Fig. 1b) and reproductive structures present (Frederiksen and Odvody 2000), the cause of disease lesions was likely *Colletotrichum sublineola* Henn. ex Sacc. & Trotter 1913 (causal agent of anthracnose).

Effects of *bmr* on insects in laboratory feeding assays using field-grown plant tissues

Larval feeding on *bmr6*, *bmr12*, and WT leaf tissue was examined in laboratory assays. Between the field observations and laboratory assays, similar feeding damage trends were generally noted for sampling dates in the same year, on sample dates where field damage on all lines occurred. For example, no significant differences were noted for feeding damage at any sample date in 2011, or in laboratory assays on day 2 in 2011 (Tables 1 and 3). Significantly less feeding was observed in the laboratory assays on day 2 for *bmr6* leaves compared with *bmr12* (Table 3) as well as in the first field sample date (July 24) in 2012 (Table 1). Significantly less feeding damage occurred on *bmr6* leaves compared to the other two lines when sampled in the field on August 1, 2013 (Table 1), and the same result was noted in laboratory assays (Table 3).

There was significantly lower fall armyworm damage to leaves of both *bmr6* ($F = 24.80$, $P = 0.0001$) and *bmr12* ($F = 10.10$, $P = 0.0052$) lines compared to WT on the first sample date of the laboratory assay in 2011 (Table 3). Both fall armyworms ($F = 6.58$, $P = 0.020$) and corn earworms ($F = 4.94$, $P = 0.039$) caused significantly more damage to the *bmr12* leaves compared to WT leaves on day 2 of the laboratory study in 2012 (Table 3). There was significantly less damage on *bmr6* compared to WT leaves by both corn earworms ($F = 24.92$, $P < 0.0001$) and fall armyworms ($F = 39.44$, $P < 0.0001$) on day 2, and damage to *bmr12* leaves was also generally less than that to WT leaves in 2013 laboratory assays.

Mortality was not observed when caterpillars were fed leaves collected in 2011 (Table 4). However, some caterpillars of both species died in all treatments in 2012, which indicates that leaves were more toxic in 2012 than 2011 (Table 4). Both corn earworm ($\chi^2 = 5.74$, $P = 0.016$) and fall armyworm ($\chi^2 = 4.90$, $P = 0.027$) mortality were significantly greater when they fed on *bmr6* leaves versus *bmr12* leaves in 2012. Results for 2013 were intermediate to those noted for the other years, with some mortality noted for corn earworms, which was significantly ($\chi^2 = 3.93$, $P = 0.047$) higher in *bmr12* compared to WT leaves.

Surviving larvae generally did not weigh more when fed on *bmr* compared to WT leaves in laboratory assays, but there were some exceptions. Surviving corn earworms fed *bmr12* leaves were significantly heavier than those fed leaves from *bmr6* ($F = 75.60$, $P < 0.0001$) or WT ($F = 41.9$, $P < 0.0001$) plants grown in 2011, but there

Table 2 Disease on leaves of field-grown sorghum plants

Sample date, line	Lesion % incidence	Lesion size (cm ²)	Plant death %
2011			
July 22			
Wild type	35.3a	2.8 ± 0.8a	0.0
<i>bmr6</i>	75.0b	2.8 ± 0.4a	0.0
<i>bmr12</i>	53.3ab	3.1 ± 0.6a	0.0
September 23			
Wild type	100.0a	40.5 ± 6.9a	0.0
<i>bmr6</i>	100.0a	14.2 ± 1.3b	0.0
<i>bmr12</i>	100.0a	48.8 ± 6.0a	16.0
2012			
July 24			
Wild type	20.0a	ND	0.0
<i>bmr6</i>	0.0b	ND	0.0
<i>bmr12</i>	16.7a	ND	0.0
August 6			
Wild type	29.2a	1.3 ± 0.2a	0.0
<i>bmr6</i>	8.3a	2.0 ± 0.0a	0.0
<i>bmr12</i>	19.2a	1.8 ± 0.4a	0.0
August 22			
Wild type	45.8a	3.1 ± 0.6a	0.0
<i>bmr6</i>	40.0a	2.8 ± 0.7a	0.0
<i>bmr12</i>	32.0a	6.4 ± 1.2b	0.0
2013			
July 19			
Wild type	50.0a	10.6 ± 2.1a	ND
<i>bmr6</i>	31.8a	4.0 ± 1.0a	ND
<i>bmr12</i>	52.6a	5.6 ± 1.3a	ND
August 1			
Wild type	25.0a	1.3 ± 0.2a	1.0 ± 0.0a
<i>bmr6</i>	38.5a	4.3 ± 0.5ab	1.5 ± 0.2ab
<i>bmr12</i>	37.5a	28.2 ± 5.4b	3.9 ± 0.7b
August 14			
Wild type	37.5a	15.3 ± 4.9ab	4.3 ± 0.5a
<i>bmr6</i>	54.5a	5.8 ± 0.9a	2.6 ± 0.4b
<i>bmr12</i>	54.5a	26.5 ± 6.6b	5.8 ± 1.0a

Damage ratings are in mean ± standard errors. Lesion incidence refers to the percentage of plants with any lesions. The values of like sample dates followed by different letters are significantly different at $P < 0.05$ by χ^2 analysis (percent incidence) or analysis of variance (lesion length ratings)

ND not determined

were no significant differences for fall armyworm weights (Table 4). Both surviving corn earworms and fall armyworms fed 2012 WT leaves weighed less than their counterparts fed leaves grown in 2011. Both insect species weighed significantly more when fed on *bmr12* leaves grown in 2012 compared to those fed 2012 *bmr6* leaves (Table 4). In 2013, both corn earworm and fall armyworm

larvae fed both types of *bmr* leaves weighed significantly less than those fed wild-type leaves. In 2011, corn earworms ate less of the *bmr12* leaves compared to WT, but weighed significantly more than those fed WT leaves. In contrast, corn earworms and fall armyworms ate significantly more of the *bmr12* leaves compared to WT, and weighed significantly more than caterpillars fed WT leaves in 2012. Reduced feeding was associated with reduced weights of both species of caterpillars in 2013.

Mortality of corn earworms and fall armyworms fed pith of both the *bmr6* and *bmr12* plants was significantly higher relative to WT pith in 2011 in the laboratory assays (Table 4). In 2012, only the *bmr6* pith caused significantly higher mortality to both corn earworms ($\chi^2 = 19.73$, $P < 0.0001$) and fall armyworms ($\chi^2 = 8.73$, $P = 0.0031$) as compared to WT pith. In 2013, mortality of corn earworm larvae was significantly ($\chi^2 = 4.58$, $P = 0.032$) higher for those fed *bmr12* compared to WT pith, and a similar trend was noted for *bmr6* pith. Mortality of both species of caterpillars fed the *bmr6* pith was much higher in 2011 than 2012 or 2013. Weights of survivors were not determined, because the extensive cannibalism among the larva resulted in highly variable sizes and numbers of surviving larva.

Discussion

Effects of *bmr* on insects

No consistent increased susceptibility for either *bmr* line to the insect species tested was observed relative to WT. Instead, there was often evidence of increased resistance in *bmr6* lines compared to WT. However, increased susceptibility of *bmr12* plant tissues compared to WT to both insects (field and laboratory) and disease (field) was observed in a limited number of cases. Variability due to environmental factors that affect plant growth, development and physiology, or abiotic stresses that triggered plant defense responses was observed, but overall the trend for no enhanced susceptibility to insect pests in *bmr* compared to WT was relatively consistent.

Although the level of mortality differed for caterpillars fed on pith from sorghum plants grown in different years, the resistance of the pith from the *bmr* lines relative to WT was not unexpected as this result had been observed with laboratory-grown plants (Dowd and Sattler 2015). Antibiosis has been previously reported as a resistance mechanism in sorghum lines resistant to fall armyworms (Lordello et al. 1980). This type of response has been reported in studies examining fall armyworm resistance of switchgrass (*Panicum virgatum* L.) lines with varying levels of lignin as well (Dowd et al. 2013). Transgenic

Table 3 Sorghum leaf damage by corn earworms and fall armyworms when caged with field-grown leaf pieces in laboratory assays

Year	Corn earworm Feeding (mm ²)	Corn earworm Feeding (mm ²)	Fall armyworm Feeding (mm ²)	Fall armyworm Feeding (mm ²)
Line	Day 1	Day 2	Day 1	Day 2
2011				
Wild type	24.5 ± 1.4a	81.2 ± 8.8a	46.7 ± 2.4a	142.0 ± 6.0a
<i>bmr6</i>	20.8 ± 1.7a	75.6 ± 6.8a	32.7 ± 1.4b	139.2 ± 8.8a
<i>bmr12</i>	24.8 ± 2.2a	66.0 ± 6.0a	37.1 ± 1.8b	146.8 ± 6.4a
2012				
Wild type	1.1 ± 0.4a	1.4 ± 0.4a	11.1 ± 1.4a	22.4 ± 2.5a
<i>bmr6</i>	1.6 ± 0.4ab	3.5 ± 0.8b	13.7 ± 2.0a	24.2 ± 2.5a
<i>bmr12</i>	3.1 ± 1.0b	8.8 ± 3.2b	17.9 ± 3.0a	39.8 ± 6.0b
2013				
Wild type	22.9 ± 2.4a	34.0 ± 3.2a	27.1 ± 2.5a	58.9 ± 5.2a
<i>bmr6</i>	7.2 ± 0.9b	16.0 ± 1.6b	10.5 ± 0.8b	25.4 ± 1.4b
<i>bmr12</i>	16.4 ± 1.8c	26.4 ± 2.6a	19.2 ± 2.4c	38.4 ± 4.0c

The values are means ± standard errors. The values followed by different letters in like assays are significantly different at $P < 0.05$ by analysis of variance

aspen with lowered lignin levels also had significantly less insect damage compared to untransformed aspen (Brodeur-Campbell et al. 2006).

Results of the present study suggest that the disruptions to monolignol biosynthesis found in either of the *bmr* lines may alter resistance to corn earworms through altered nutritional effects. Reduced feeding on *bmr12* leaves grown in 2011 compared to WT leaves occurred, but larvae fed *bmr12* leaves were heavier. Larvae ate more of the *bmr12* leaves grown in 2012 than the WT, and larvae fed *bmr12* leaves were again heavier. Fall armyworms responded differently than corn earworms. In some cases, fall armyworm leaf feeding rates were similar for WT and *bmr* lines, but weights of larvae fed on *bmr* lines were significantly less than those fed on WT. In other cases, fall armyworm leaf feeding damage to *bmr* lines was significantly greater than on WT, but weights of survivors were not significantly different. Both of these situations suggest that the *bmr* lines were less nutritious than WT due to altered secondary metabolite composition that interfered with nutrient availability, and compensatory feeding was occurring. Compensatory feeding is known to occur in fall armyworms when artificial diet is diluted with nonnutritional materials, such as cellulose (Wheeler and Slansky 1991). The reduced feeding associated with increased weights observed for corn earworms on the 2011 leaves suggests that the *bmr12* leaves were more nutritious than WT ones, which may be consistent in a tissue that had reduced levels of nonnutritional components such as lignin. In younger switchgrass plants, increased feeding was associated with reduced levels of lignin (Dowd et al. 2013). However, factors other than lignin content or its composition may be more important in determining overall insect

resistance, depending on the age of the plants (Dowd et al. 2013). This phenomenon may also have occurred in previous (Dowd and Sattler 2015) and in the present study, where pith from *bmr* plants was often more toxic than pith from WT plants to both insect species when tested in the laboratory.

In the present study, differing trends in incidence and damage or size of populations were noted between sucking versus chewing insects. Differential species responses to plants with different levels of resistance have also been reported in other studies of sorghum insect resistance. When resistant and susceptible sorghum lines were examined for the type of resistance involved, antibiosis was noted for *Chilo partellus* (Swinhoe) (Lepidoptera Pyralidae), and antixenosis was noted for *Busseola fusca* (Fuller) (Lepidoptera Noctuidae) (Van den Berg et al. 1994). Both antibiosis and antixenosis are also involved in sorghum resistance to aphids (Huang et al. 2013). Dhurrin, a cyanogenic glucoside, which is synthesized in response to drought (Duncan 1996), may contribute to resistance in some sorghum lines to the southwestern corn borer *Diatraea grandiosella* Dyar (Lepidoptera: Pyralidae) (Cheng et al. 2013) and fall armyworm (Krothapalli et al. 2013), while higher jasmonic acid biosynthesis may contribute to resistance to other insects (Cheng et al. 2013). Dhurrin is derived from the aromatic amino acid tyrosine. Because both *bmr6* and *bmr12* mutations disrupt genes involved in phenylpropanoid metabolism, more phenylalanine or tyrosine may be available for the synthesis of defensive compounds such as dhurrin. Previous reports on the same lines used in the present study indicate reduced lignin in the two *bmr* lines. (Sattler et al. 2009) and increased levels of phenolics such as ferulic acid and vanillic acid (Palmer

Table 4 Corn earworm survivorship (percent mortality) and growth (weight) when caged with field-grown leaf and pith pieces in laboratory assays

Tissue, year Line	Corn earworms % Mortality	Corn earworms Weight (mg)	Fall armyworms % Mortality	Fall armyworms Weight (mg)
Leaves, 2011				
Wild type	0.0a	0.13 ± 0.01a	0.0a	0.35 ± 0.1a
<i>bmr6</i>	0.0a	0.11 ± 0.01a	0.0a	0.36 ± 0.1a
<i>bmr12</i>	0.0a	0.19 ± 0.01b	0.0a	0.35 ± 0.1a
Leaves, 2012				
Wild type	30.0ab	0.11 ± 0.01ab	8.2ab	0.10 ± 0.01a
<i>bmr6</i>	45.4b	0.09 ± 0.01a	10.6a	0.10 ± 0.01a
<i>bmr12</i>	23.1a	0.12 ± 0.01b	2.4b	0.14 ± 0.01b
Leaves, 2013				
Wild type	9.1a	0.13 ± 0.01a	0.9a	0.21 ± 0.01a
<i>bmr6</i>	14.1ab	0.10 ± 0.01b	0.0a	0.11 ± 0.01b
<i>bmr12</i>	20.5b	0.10 ± 0.01b	0.9a	0.13 ± 0.01b
Pith, 2011				
Wild type	5.6a	ND	22.2a	ND
<i>bmr6</i>	51.3b	ND	64.7b	ND
<i>bmr12</i>	36.6b	ND	63.6b	ND
Pith, 2012				
Wild type	3.4a	ND	4.8a	ND
<i>bmr6</i>	25.9b	ND	13.7b	ND
<i>bmr12</i>	7.2a	ND	1.9a	ND
Pith, 2013				
Wild type	10.0a	ND	2.1a	ND
<i>bmr6</i>	20.6ab	ND	1.0a	ND
<i>bmr12</i>	23.8b	ND	0.0a	ND

The values for weights are means ± standard errors. The values of like assays followed by different letters are significantly different by χ^2 (mortality) or analysis of variance (survivor weights)

ND = not determined

et al. 2008). When incorporated into artificial diets, larvae fed vanillic acid at levels approximating those found in *bmr* lines were smaller (Dowd and Sattler 2015).

Effects of *bmr* on disease

Considerable differences in resistance, as indicated by leaf lesion incidence and size, were noticed, from 2011 to 2013, and much smaller lesions were observed during 2012 and 2013. Prior studies of stalk inoculations have indicated enhanced resistance to *Fusarium thapsinum* Klittich, Leslie, Nelson, Marasas, *F. verticillioides* (Sacc.) Nirenberg, or *Alternaria alternata* (Fries) Keissler in *bmr6* and/or *bmr12* sorghum compared to WT isolines under controlled conditions (Funnell-Harris et al. 2010). Field studies of grain infections also demonstrated reduced incidences of *Fusarium* sorghum pathogens in *bmr* lines (Funnell-Harris et al. 2010). In the current field study, reduced lesion damage by anthracnose was more frequently observed in *bmr6* than *bmr12* lines compared to wild type for all years of the study. Anthracnose has many pathotypes (Moore

et al. 2010) so it may be possible that different pathotypes were involved each year, although in a particular area the same pathotype usually occurs from year to year (Tesso et al. 2012).

Reducing lignin content in wheat led to increased susceptibility to *Blumeria graminis* DC f. sp. *tritici* Ém Marchal (Bhuiyan et al. 2009b), while reduced lignin levels were associated with greater resistance to *Sclerotinia sclerotiorum* (Lib. DeBary) in soybeans *Glycine max* (L.) (Peltier et al. 2009). In sorghum, some fungal species induce the synthesis of colored pigments, 3-deoxyanthocyanins, and these compounds may deter fungal colonization (Lo et al. 1996). These same compounds, which are also derived from phenylpropanoid metabolism, may occur at higher levels in the *bmr* lines in response to stresses. It was previously postulated that enhanced production of these compounds due to accumulation of phenylpropanoid precursors in *bmr6* and *bmr12* was one possible mechanism for increased resistance of these lines to fungi (Funnell-Harris et al. 2010). Enhanced production of the defense signaling molecule salicylic acid may also be responsible

for enhanced fungal resistance in the *bmr6* and *bmr12* lines (Funnell-Harris et al. 2010). Enhanced salicylic acid production would also promote enhanced aphid resistance based on prior studies (Koornneef and Pieterse 2008). However, upregulated salicylate production is typically associated with downregulated jasmonic acid production (Koornneef and Pieterse 2008) and antagonistic communication between these two pathways has also been reported (Li et al. 2004). This would be inconsistent with the enhanced caterpillar resistance of the *bmr* lines often noted in the present study since upregulated jasmonic acid production is also typically associated with enhanced resistance to caterpillars (Sun et al. 2011). Thus, there is evidence that resistance to different insect feeding groups and pathogen types may not follow the same trends.

Year-to-year variation

Differences in abiotic conditions appeared to affect responses of insects and disease in the field, and of insects to field-grown tissues in laboratory assays. In 2012, reduced amounts of leaf damage on individual plants (when it occurred) by insects in the field were observed, and less insect damage of leaves grown in this year in laboratory assays for all lines compared to 2011 and 2013, but the opposite effect was observed for pith feeding assays. The year 2012 was hotter and drier than 2011 and 2013, which had relatively normal temperatures and rainfall amounts (www.weather.gov/index.php?wfo=ilx). Abiotic factors can also induce the expression of genes that respond to biotic stresses (Atkinson and Urwin 2012). High temperatures can increase the resistance of plants to plant pathogens (Maimbo et al. 2007) as well as increase the levels of defensive compounds active against both insects and plant pathogens (Djanaguiraman et al. 2011; Mosolove and Valueva 2011). However, higher temperatures can result in decreased levels of some defensive compounds in other instances (Kuokkanen et al. 2001), and may have affected lignin biosynthesis in the present study. Temperature or other abiotic stresses can also alter plant susceptibility to insects and pathogens to different degrees for different plant species (Roy et al. 2004) and varieties of the same species (Grinnan et al. 2013). It is possible that abiotic factors may negatively affect the insect pest or pathogen directly, or indirectly through biotic factors influencing insects or disease.

Conclusions

The present study indicated that sorghum lines *bmr6* and *bmr12* generally do not have increased susceptibility to insects and disease as compared to WT. There were some

instances where *bmr12* leaves were more damaged by insects or disease as compared with WT, suggesting that interactions of genotype and environmental factors may affect outcomes. On the other hand, as noted in prior laboratory assays (Dowd and Sattler 2015) there were several instances where damage to *bmr6* leaves was less than that to WT. These results suggest that this line may have enhanced resistance under certain circumstances, and might be more useful than *bmr12* to reduce lignin for sustainable production of biomass for bioenergy uses. This information suggests that larger scale field trials would be appropriate to further evaluate their potential. Chemical and molecular analyses of the potential resistance factors of the pith of the *bmr* lines are in progress and would be of value in monitoring for transfer of useful resistance mechanisms during the breeding process.

Author contribution statement

All authors conceived and designed the research. SS developed the sorghum lines. PD conducted experiments and analyzed the data. DF provided expertise in disease diagnostics. PD wrote the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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