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Heliooverpa zea (Lepidoptera: Noctuidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae) Responses to Sorghum bicolor (Poales: Poaceae) Tissues From Lowered Lignin Lines

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Subject Editor: John Palumbo

J. Insect Sci. 15(2): 2015; DOI: 10.1093/jisesa/ieu162

There is global interest in developing fuels for the transportation sector from renewable resources as a means to reduce dependency on petroleum and other finite fuel sources. One of the impediments to converting biomass to biofuels is the presence of the cell wall polymer lignin, which interferes with the release of sugars from the corresponding cell wall polysaccharides, cellulose, and hemicelluloses during enzymatic saccharification (Dien et al. 2009). Therefore, reducing lignin levels through traditional breeding or genetic engineering is a target for bioenergy feedstock improvement but reducing lignin in cell walls may impair pest resistance where lignin is a major component of resistance.

There are mutants of many plant species that have altered lignin content and subunit composition. The brown midrib phenotype has long been associated with reduced lignin content of maize, Zea mays L. (referred to as bm), and sorghum, Sorghum bicolor (L.) Moench (Poales: Poaceae) (referred to as bmr) (Sattler et al. 2010). Although bm plants have reduced lignin and improved forage digestibility, maize bm plants can have increased stalk breakage caused by insect damage or pathogens when compared with wild-type lines (Bärré and Aguilier 1993). However, brown midrib mutants of other grass species are acceptable agronomically (Pedersen et al. 2005). The sorghum bmr6 phenotype is due to a nonsense mutation that causes premature truncation of caffeic O-methyl transferase (COMT). The bmr12 plants have reduced lignin levels, and syringyl subunits within lignin are greatly reduced relative to other subunits compared with wild-type plants (Bout and Vermerris 2003, Palmer et al. 2008). These two bmr mutants have increased ethanol conversion efficiency compared with wild-type (Dien et al. 2009). However, the effects of these two mutations on pest resistance have not been examined. On the basis of prior work with switchgrass (Dowd et al. 2013), we hypothesized that the bmr6 and bmr12 sorghum lines will retain insect resistance compared with the near isogenic wild-type line.

Here, we report on studies with corn earworms, Heliooverpa zea (Boddie) and fall armyworms Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), two sorghum major pests. Younger bmr leaves had significantly greater feeding damage in some assays than wild-type leaves, but older bmr6 leaves generally had significantly less damage than wild-type leaves. Caterpillars feeding on the bmr6 leaves often weighed significantly less than those feeding on wild-type leaves, especially in the S. frugiperda assays. Larvae fed the pith from bmr stalks had significantly higher mortality compared with those larvae fed on wild-type pith, which suggested that bmr pith was more toxic. Thus, reducing lignin content or changing subunit composition of bioenergy grasses does not necessarily increase their susceptibility to insects and may result in increased resistance, which would contribute to sustainable production.

Key Words: resistance, agricultural entomology, plant resistance

ABSTRACT. The presence of lignin within biomass impedes the production of liquid fuels. Plants with altered lignin content and composition are more amenable to lignocellulosic conversion to ethanol and other biofuels but may be more susceptible to insect damage where lignin is an important resistance factor. However, reduced lignin lines of switchgrasses still retained insect resistance in prior studies. Therefore, we hypothesized that sorghum lines with lowered lignin content will also retain insect resistance. Sorghum excised leaves and stalk pith Sorghum bicolor (L.) Moench (Poales: Poaceae) from near isogenic brown midrib (bmr) 6 and 12 mutants lines, which have lowered lignin content and increased lignocellulosic ethanol conversion efficiency, were examined for insect resistance relative to wild-type (normal BTx623). Greenhouse and growth chamber grown plant tissues were fed to first-instar larvae of corn earworms, Heliooverpa zea (Boddie) and fall armyworms Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), two sorghum major pests. Younger bmr leaves had significantly greater feeding damage in some assays than wild-type leaves, which suggested that the bmr6 leaves gener-

Materials and Methods

Insects. The H. zea and S. frugiperda were obtained from colonies rear ed on pinto bean-based diet at 27 ± 1°C, 50 ± 10% relative humidity (RH), and a photoperiod of 14:10 (L:D) h, as described previously (Dowd 1988). First instars without any prior feeding experience were randomly selected and used in all assays.

Plant Phenolic Bioassays. The plant phenolics adipic acid (99%), p-coumaric acid (≥98.0%), ferulic acid (99%), sinapic acid (≥98%), syringic acid (≥95%), and vanillic acid (97%) were obtained from...
Sigma/Aldrich (St. Louis, MO) (www.sigmaaldrich.com). They were incorporated into warm, still liquid pinto bean diets by blending with a vortex mixer as described previously (Dowd 1988). Phenolic acids were incorporated into diet at concentrations reported previously from near isogenic wild-type, bmr6, and bmr12 RTx430 stalks (Palmer et al. 2008) as follows for compounds listed above in order, in µg/g wet weight: “wild-type” was 67.8, 30.0, 8.8, 0.0, 5.6, and 17.7; “bmr6” was 36.1, 84.0 42.8, 13.8, 21.0, and 9.3; and “bmr12” was 51.5, 30.1, 21.1, 4.8, 3.2, and 20.7.

Leaf disk diets were prepared by substituting wild-type leaf tissue (12 leaf stage plants; fourth leaf from the top) for pinto beans, wheat germ, and brewer’s yeast and a proportional amount of water from the pinto bean diet (Dowd 1987) to provide similar nutritional composition as leaves for examining the effects of added phenolics under nutrient stress conditions. Diet was dispensed between two metal plates spaced 1 mm apart, cut into 4-mm disks when firm and freeze dried (Dowd et al. 2011). Because the wild-type stalks already contained some phenolics at higher levels than the bmr lines, only those phenolics that were at a higher concentration in the bmr lines were added to the disks, and only those phenolics that had levels at least 10% higher in the bmr compared with wild-type plants were used. For the “bmr6”-simulated leaf disks, ferulic, vanillic, sinapic, and syringic acids were added at 34.0, 34.0, 13.8, and 16.0 µg/g, respectively. For the “bmr12”-simulated disks, only vanillic acid was added at 12.3 µg/g wet weight. For the “wild-type”-simulated disks, no additional phenolic acids were added (only solvent control). Freeze-dried disks were used as described previously (Dowd et al. 2011). The phenolics acids were added in acetone to each dry disk, the acetone was evaporated in a chemical fume hood for 30 min, and the diet disks were rehydrated with sterile distilled water.

Each treatment of the pinto bean diet was cut into pieces sufficient for ad libitum feeding, and each piece was placed in a separate well of a 24-well tissue culture plate (Dowd 1988). An individual larva was added to each well. For leaf disk assays, rehydrated sorghum leaf disk materials were placed on a Teflon disk inside a Petri dish containing 3% water agar. Ten first-instar larvae were added to each dish. The surviving larvae were weighed after 5 d (pinto bean diet pieces) or 3 d (sorghum leaf disks).

**Plants.** Wild-type, bmr6, and bmr12 near isogenic plants in the background BTx623 were used in the present experiments. The near isogenic lines containing the bmr6 and bmr12 alleles were previously developed by crossing source of the bmr6 or bmr12 allele with BTx623 and four generations of backcrossing to BTx623 (Pedersen et al. 2006). When plants were grown in the Peoria greenhouse or a growth room (Peoria only), pots containing a previously reported soil and fertilizer mix, which contained bark mix (Dowd et al. 2006). The phenolics acids were added in acetone to each dry disk, the acetone was evaporated in a chemical fume hood for 30 min, and the diet disks were rehydrated with sterile distilled water.

Each treatment of the pinto bean diet was cut into pieces sufficient for ad libitum feeding, and each piece was placed in a separate well of a 24-well tissue culture plate (Dowd 1988). An individual larva was added to each well. For leaf disk assays, rehydrated sorghum leaf disk materials were placed on a Teflon disk inside a Petri dish containing 3% water agar. Ten first-instar larvae were added to each dish. The surviving larvae were weighed after 5 d (pinto bean diet pieces) or 3 d (sorghum leaf disks).

**Leaf tissue bioassays.** All bioassays were performed in Peoria. Leaf section bioassays were performed using Petri dishes with tight fitting lids as described previously (Dowd et al. 2007). Immature leaves (2nd from top, 12 cm of the leaf including the tip) of five leaf plants and more mature leaves (4th from top, 20 cm of the leaf including the tip) of 10–12 leaf stage plants were used in the bioassays. After 2 d, additional 4 cm long leaf pieces were removed from the initial harvest point of the 10–12 leaf plants to investigate wounding induced factors. Approximately 2 cm2 leaf pieces were used in the assays, which were removed 0–6 cm from the leaf base, except for the additional piece of 4-cm long piece removed 2 d later from previous harvest site. There was sufficient leaf material for ad libitum feeding for the duration of each experiment. Leaf pieces were placed in Petri dishes with tight fitting lids, containing moistened filter paper, and 10 newly hatched first-instar caterpillars without prior feeding experience were added to each dish.

For pith assays, stalk sections were harvested from growth room grown plants after other assays were completed. The plants were post-flowering, at soft dough stage of grain filling, and the stalk were green and turgid. Approximately 1 cm in diameter internodes between the first and second leaf from the top were harvested, which had color differences in the pith amongst the respective lines. Approximately 2-cm-long pith sections were placed in the Petri dishes as described for leaf piece bioassays with 10 first-instar caterpillars.

Feeding damage on leaves was evaluated by determining the total number of 0.25 mm2 or 1 mm2 hole equivalents after 2 d as described previously (Dowd et al. 2007, 2011); caterpillars often molled to the second instar during that period. Because little mortality occurred in leaf assays, survivors were weighed using a Mettler AE104 analytical balance (Mettler Instrument Corp., Highstown, NJ), which is accurate to 0.01 mg (Dowd et al. 2007, 2011). For the pith assays, insect feeding damage was not determined due to the difficulty of quantifying varying lengths and depths of feeding damage within the pith sections. Only mortality was determined after a 3-day feeding period due to highly variable weights resulting from cannibalism of dead larvae.

**Statistical Analysis.** Statistically significant differences in overall insect mortality for each main effect plant variety treatment were determined by Chi square analysis, and differences in feeding rates and weights of survivors were determined by analysis of variance. SAS Proc Freq was used for Chi square analysis, and SAS Proc GLM was used for the other analyses. Windows Version 8.0 of the SAS software (SAS Institute 1999) was used.

**Results.** Consistent with our hypothesis, both first-instar H. zeas- and S. frugiperda-fed sorghum leaves at different plants stage generally did not show statistically significant differences (P < 0.05) in feeding damage, mortality (very limited, so data not shown), or survivor weights indicative of reduced resistance for bmr compared with wild-type lines after 2 d of feeding on leaves (Table 1, Supp Table S1 [online only]). However, there was one exception; H. zeas-fed leaves from five-leaf stage bmr12 plants; leaves from this plant stage had significantly (P < 0.01) greater feeding damage (mm2) relative to wild-type leaves in both experiments (F = 21.08, P < 0.001, df = 1, 19 and F = 14.06, P = 0.001, df = 1,23) (Table 1). In contrast, there were several cases where the leaves of mutant lines were significantly more resistant to one or both of the insect species, based on significantly lower amounts of leaf damage or lower weights of survivors (Table 1). The leaves from both bmr6 5 leaf and 10–12 leaf plants had significantly less (P < 0.05 or P < 0.01) feeding damage from S. frugiperda compared with wild-type leaves in nearly all experiments. The amounts of S. frugiperda feeding damage on bmr12 leaves were not significantly different from wild-type leaves. Similar results were obtained whether plants were grown in the greenhouse or plant growth room, although some variation of some results was noted between the first and second experiments for some components.

Damaging the leaves mechanically did not change relative resistance trends for the S. frugiperda that were observed for bmr versus wild-type undamaged leaves. Results with the H. zeas were more variable but significantly (P < 0.05 or 0.01) less leaf material was removed from bmr6 leaves than wild-type leaves in several cases, which indicated bmr6 leaves were more resistant. For one experiment, in contrast to observations from first assay, when another piece was removed from
Table 1 Sorghum leaf effects on *H. zea* and *S. frugiperda* larvae

|                | H. zea feeding (mm²) | Weight (mg) | S. frugiperda feeding (mm²) | Weight (mg) |
|----------------|----------------------|-------------|-----------------------------|-------------|
| Ten-leaf plant, mature leaf (greenhouse-Lincoln, NE) | Wild-type | 53 ± 2a | 0.19 ± 0.01b | 137 ± 9b | 0.47 ± 0.02b |
|                | bmr6 | 53 ± 2a | 0.19 ± 0.01b | 143 ± 10b | 0.47 ± 0.02b |
|                | bmr12 | 54 ± 2a | 0.19 ± 0.01b | 126 ± 9ab | 0.48 ± 0.02ab |
| Ten-leaf plant, mature leaf (greenhouse-Peoria, IL) | Wild-type | 25 ± 2a | 0.17 ± 0.01b | 53 ± 2b | 0.61 ± 0.02b |
|                | Bmr6 | 25 ± 2a | 0.17 ± 0.01b | 53 ± 2b | 0.61 ± 0.02b |
|                | Bmr12 | 29 ± 1a | 0.22 ± 0.01a | 63 ± 1a | 0.72 ± 0.03a |
| Five-leaf plant, immature leaf (growth room) experiment 1 | Wild-type | 39 ± 1a | 0.21 ± 0.01a | 68 ± 2a | 0.35 ± 0.01a |
|                | bmr6 | 48 ± 2b* | 0.23 ± 0.01a | 54 ± 2b* | 0.31 ± 0.01b* |
|                | bmr12 | 50 ± 2b* | 0.23 ± 0.01a | 70 ± 1a | 0.34 ± 0.01a |
| Five-leaf plant, immature leaf (growth room) experiment 2 | Wild-type | 32 ± 1a | 0.36 ± 0.01a | 42 ± 1a | 0.46 ± 0.02a |
|                | bmr6 | 29 ± 1b | 0.32 ± 0.02ab | 35 ± 1b | 0.37 ± 0.01b* |
|                | bmr12 | 38 ± 1c | 0.31 ± 0.02b | 42 ± 2a | 0.52 ± 0.02a |
| Twelve-leaf plant, mature leaf (growth room) experiment 1 | Wild-type | 48 ± 3a | 0.24 ± 0.01a | 59 ± 3a | 0.37 ± 0.01a |
|                | bmr6 | 41 ± 1b | 0.21 ± 0.01b | 44 ± 2b* | 0.42 ± 0.02b |
|                | bmr12 | 43 ± 4ab | 0.20 ± 0.01b* | 59 ± 2a | 0.34 ± 0.01c* |
| Twelve-leaf plant, mature leaf (growth room) experiment 2 | Wild-type | 29 ± 1a | 0.26 ± 0.01a | 65 ± 3a | 0.39 ± 0.02a |
|                | bmr6 | 25 ± 1b | 0.17 ± 0.01b | 53 ± 2b | 0.30 ± 0.02b* |
|                | bmr12 | 29 ± 1a | 0.27 ± 0.02a | 63 ± 1a | 0.37 ± 0.02a |
| Twelve-leaf plant, mature leaf—recut (growth room) experiment 1 | Wild-type | 56 ± 3a | 0.23 ± 0.01a | 71 ± 4a | 0.42 ± 0.02a |
|                | bmr6 | 45 ± 3b* | 0.21 ± 0.01ab | 58 ± 3b* | 0.36 ± 0.01b* |
|                | bmr12 | 41 ± 3b* | 0.20 ± 0.01b | 61 ± 4ab | 0.38 ± 0.02ab |
| Twelve-leaf plant, mature leaf—recut (growth room) experiment 2 | Wild-type | 29 ± 2a | 0.21 ± 0.01a | 38 ± 2a | 0.48 ± 0.02a |
|                | bmr6 | 18 ± 1b | 0.16 ± 0.01b* | 29 ± 2b | 0.33 ± 0.01b* |
|                | bmr12 | 29 ± 1a | 0.24 ± 0.01a | 42 ± 2a | 0.55 ± 0.03a |

At least 10 leaves of each line were used. Mean ± standard error values reported are in mm² (feeding) and mg (weights) after 2 d of feeding. Values in columns for the same experiment followed by different letters are significantly different by Chi square analysis. Values of bmr (low lignin) lines in columns for the same experiment followed by a “*” are significantly different from wild-type (normal lignin) values at *P* < 0.01.

Table 2. Sorghum stalk pith effects on *H. zea* and *S. frugiperda* larvae

|                | H. zea, % mortality | S. frugiperda, % mortality |
|----------------|---------------------|---------------------------|
| Experiment 1   |                      |                           |
| Wild-type      | 1.7a                | 10.4a                     |
| bmr6           | 36.4b*              | 27.7b                     |
| bmr12          | 29.96*              | 25.5b                     |
| Experiment 2   |                      |                           |
| Wild-type      | 6.4a                | 2.7a                      |
| bmr6           | 47.3b*              | 21.2b*                    |
| bmr12          | 37.3b*              | 34.0b*                    |

Pith from at least eight plants of each line was used. Values followed by different letters for the same experiment are significantly different at *P* < 0.05 by Chi square analysis. Values of bmr (low lignin) lines in columns for the same experiment followed by a “*” are significantly different from wild-type (normal lignin) values at *P* < 0.01.

**Discussion**

Consistent with our hypothesis, no general increased susceptibility of either bmr6 or bmr12 leaves to the insect species tested in this study were observed, although in some cases younger bmr leaves from five-leaf stage plants had more feeding damage than wild-type leaves. Interestingly, there were several instances where bmr leaves and pith were more resistant to these larvae compared with the wild-type tissues based on higher mortality (pith) or lower amounts of feeding (leaves). The enhanced resistance was especially evident for *S. frugiperda*-larvae-fed bmr6 tissue. Variations between experiments may have been due to environmental factors (temperatures and light intensity in the different growth locations) or subtle differences plant developmental stage. Genotype by environment effects can influence agronomic traits in *brown midrib* lines (Cassler et al. 2003, Palmer et al. 2008), which can also influence insect resistance (Dowd and Johnson 2009). The relative trend for resistance was generally consistent whether the insects fed on undamaged or previously damaged leaves, but we cannot rule out that the relationship may change over time due to resistance factors induced specifically by insect feeding, which can vary depending on the insect species involved (Rodriguez-Saona et al. 2010). However, based on reports in maize (Shen et al. 2000), the time frame we used for the assays should have been sufficient to observe induced responses.

In some cases, feeding damage to leaves was similar between wild-type and bmr lines, but postfeeding weights were significantly less for larvae-fed bmr leaves compared with larvae-fed wild-type leaves. In other cases, feeding damage on bmr leaves was significantly greater than on wild-type leaves, but weights of survivors were not significantly different. Both of these situations suggest that the bmr lines were less nutritious than the wild-type, and compensatory feeding was
The same unknown factors could be present in both bmr lines, and these same factors could be responsible for the increased mortality, reduced leaf damage, and lower larval weights in the pith and leaf feeding assays, respectively. Considering that monolignol pathway is active in both leaves and stalks, and bmr6 and bmr12 both affect this pathway, the accumulation of phenolic compounds resulting from alteration to this pathways may partly play a role in resistance. The studies with diets involving added phenolic acids that simulated levels observed in bmr stalks (Table 3) suggest that changes in phenolic composition found in bmr mutants may increasing toxicity or interfering with nutrient absorption. No significant differences in insect weights were noted for experiments with nutritionally complete pinto bean based artificial diet was used, but weights of both H. zea and S. frugiperda were lower when they fed on the simulated "bmr" compared with "wild-type" diet made from sorghum leaf material. This information suggests that diet nutrient composition influences the level of resistance conferred by potential resistance molecules. No effect was observed with the nutritionally rich pinto bean diet, but a significant effect was detected with the nutritionally poor sorghum leaf diet, which suggests the phenolic acids are interfering with nutrient absorption. S. frugiperda caterpillars that fed upon the simulated "bmr6" and "bmr12" leaf diet disks weighed less than those fed the "wild-type" leaf diet disks. However, the "bmr12" diet disks only had additional valeric acid, whereas the "bmr6" leaf diet also had ferulic and syringic acid added. This result suggests that increased levels of valinic acid may be associated with increased insect resistance in the bmr lines. Stem and pith resistance to the stalk borer, Sesamia nonagrioides (Lefebvre) in several lines of maize was correlated with p-coumaric content but not with several other phenolics, including ferulic, sinapic, syringic, and valinic acids (Santiago et al. 2005). Syringic and valinic acid, but not ferulic or p-coumaric acid concentration were correlated with p-coumaric content but not with several other phenolics, including ferulic, sinapic, syringic, and valinic acids (Santiago et al. 2005). Syringic and valinic acid, but not ferulic or p-coumaric acid concentration was correlated with p-coumaric content but not with several other phenolics, including ferulic, sinapic, syringic, and valinic acids (Santiago et al. 2005). Syringic and valinic acid, but not ferulic or p-coumaric acid concentration was correlated with p-coumaric content but not with several other phenolics, including ferulic, sinapic, syringic, and valinic acids (Santiago et al. 2005). Syringic and valinic acid, but not ferulic or p-coumaric acid concentration was correlated with p-coumaric content but not with several other phenolics, including ferulic, sinapic, syringic, and valinic acids (Santiago et al. 2005).

Table 3. Altered phenolic diet effects simulating concentrations in sorghum stalks on H. zea and S. frugiperda larvae

| Diet Type               | H. zea % mortality | Weight (mg) | S. frugiperda % mortality | Weight (mg) |
|------------------------|--------------------|-------------|---------------------------|-------------|
| Artificial pinto bean-based diet |                   |             |                           |             |
| Solvent control        | 0.0a               | 6.3 ± 0.3a  | 0.0a                      | 4.6 ± 0.2a  |
| "Wild-type"            | 0.0a               | 6.7 ± 0.4a  | 0.0a                      | 4.4 ± 0.4a  |
| "bmr6"                 | 0.0a               | 6.8 ± 0.2a  | 0.0a                      | 3.4 ± 0.3b  |
| "bmr12"                | 0.0a               | 6.1 ± 0.4a  | 0.0a                      | 3.5 ± 0.4b  |
| Sorghum leaf disk diet | "Wild-type"        | 0.0a        | 0.21 ± 0.03a              | 0.20 ± 0.01a|
|                        | "bmr6"             | 0.0a        | 0.20 ± 0.02a              | 0.13 ± 0.01b*|
|                        | "bmr12"            | 0.0a        | 0.15 ± 0.02a              | 0.13 ± 0.01b*|

Values are after 3 d for leaf disk diets and 5 d for artificial diet. Weights are means ± standard errors in mg. Values followed by different letters for like studies are significantly different at P < 0.05 by Chi square analysis (mortality) or analysis of variance (weights). Values of "bmr" diets in columns for the same experiment followed by a "*" are significantly different from "wild-type" diet values at P < 0.01. See Materials and Methods for phenolic additions that simulate the phenolic acid compositions found in wild-type, bmr6, and bmr12 stalks (Palmer et al. 2008).

occurring in some cases, but it did not result in greater larval weights. A compensatory feeding response has also been reported when S. frugiperda were fed diet with increased levels of non-nutritional cellulose (Wheeler and Slansky 1991). In some cases, resistance was due to antibiosis (toxic compounds), which is indicated when reduced feeding results in lower larval weights. This phenomenon was observed in several cases when the larvae fed on bmr6 compared with wild-type leaves. Lignin can be an important insect resistance factor in plants (Swain 1979), although the complex composition of lignin and interconnected metabolic network involved the synthesis of its precursors makes it difficult to predict how altering lignin concentration or composition of plants will affect insect resistance. Reduced ferulate crosslinking in fescue (Festuca sp.) resulted in increased damage by fall armyworms (Buanafina and Fescemyer 2012), which illustrates the importance of ester and ether-linked ferulic acid that are separate from lignin polymers in grass cell walls. However, low lignin lines of switchgrass (Panicum virgatum L.) (where lignin levels were reduced from ~7 to 4%) that had higher rates of saccharification and fermentation retained resistance to S. frugiperda compared with high lignin lines (Dowd and Johnson 2009, Dowd et al. 2013). Age of plants and tissues can also influence resistance levels (Smith et al. 1994), which we also observed in evaluating feeding on leaves from 5-leaved compared with 10- or 12-leaved plants. Similarly, a significant positive association was observed between degree of insect resistance and lignin levels in younger switchgrass plants but not older plants (Dowd et al. 2013). This study indicated sorghum lines with lowered lignin can result in greater resistant to insects than lines unimpaired in their ability to synthesize lignin.

Unexpectedly, the pith of both bmr lines was highly resistant relative to wild-type pith, based on higher rates of mortality observed for larvae-fed bmr pith. Previous studies have indicated impairing different steps in monolignol biosynthesis results in reddish brown to tan stem and stalk pigmentation, including the CAD and COMT mutants examined in this study (Mackay et al. 1997, Tsai et al. 1998, Sibout et al. 2005, Zhang et al. 2006). There are differences in pith and midrib (leaf vein) coloration between bmr6 and bmr12 (Porter et al. 1978, Saballos et al. 2009), probably because bmr6 and bmr12 block different steps of the monolignol biosynthetic pathway. However, the increased mortality of both H. zea and S. frugiperda on both bmr mutant pith types suggests a common chemical or biochemical resistance factor(s), yet to be identified, which is not related to differences in pith color. The greater resistance observed in bmr pith and bmr leaves at times suggest the similar phenolic compounds or pathway intermediates may accumulate in both tissues due to altered phenylpropanoid metabolisms caused by these mutations. Alternatively, the changes in phenylpropanoid metabolism could induce the expression of unrelated resistance genes not involved in this metabolic pathway. Additionally, the loss of these biosynthetic enzymes in the bmr6 and bmr12 lines may have a previously unrecognized role, which is not unprecedented. Transgenic overexpression of a peroxidase altered expression of other, unrelated defensive genes in tomato, Solanum lycopersicum L. (Suzuki et al. 2012). In addition, the lignin biosynthetic enzyme Cinnamoyl-CoA reductase (CCR) is also involved in defense signaling in rice, Oryza sativa L. (Kawasaki et al. 2006).

This study indicates that sorghum lines bmr6 and bmr12, which are easier to enzymatically saccharify, generally do not reduced levels of insect resistance. The increased resistance of bmr6 leaves and pith to insects observed suggests the bmr6 mutant may actually have sufficient enhanced insect resistance, such that it would require fewer insecticide applications in field production. This information suggests that sorghum bmr traits, which enhance conversion of lignocellulosic biomass to ethanol, or other biofuels are viable objections for sustainable bioenergy feedstock production. Further evaluations under field conditions are needed to better assess the potential of the bmr sorghum lines to be sustainably produced, for which insect resistance is an important component. The toxicity of the pith from the bmr lines to caterpillars noted in this study suggests it may promote resistance to stalk boring insects, and field studies are in progress to evaluate this potential. Chemical and molecular analyses of the potential resistance mechanisms within bmr pith are in progress. This study represents the first effort to evaluate insect resistance in sorghum lines with potential bioenergy uses. This study also provides incentive to continue investigating insect resistance of these lines under field conditions. Overall, the results of this study and other studies examining effects plants with altered lignin content and composition on insect resistance indicate that changes to this
pathway to augment biofuel production should be evaluated on a case by case basis for both the plant production and the insect resistance.

Acknowledgments

We thank B.T. Ade, Z. Demkovitch, D. Lee, and A. Maybanks (USDA-ARS, National Center for Agricultural Utilization Research) for technical assistance, D. Palmquist (USDA-ARS, Midwest Area Biometrician) for suggestions on statistical analyses, and J.F. Pedersen (USDA-ARS, University of Nebraska-Lincoln, retired), A.P. Rooney (USDA-ARS, National Center for Agricultural Utilization Research), and F.E. Vega (USDA-ARS, Beltsville Agricultural Research Center) for comments on prior versions of the manuscript. This work was supported by Agricultural Food Research Institute award number 2011-67009-30026 from the National Institute of Food and Agriculture and funding the base Agricultural Research Service projects.

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