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RESEARCH ARTICLE

Insect resistance of a full sib family of tetraploid switchgrass *Panicum virgatum* L. with varying lignin levels

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Abstract Little information is available on insect resistance mechanisms and inheritance in biomass grasses. Although reduction of lignin in biomass grasses in order to increase the efficiency of fermentation may result in increased susceptibility to insect feeding, other resistance mechanisms may be more important. Field grown leaves of two tetraploid parent (Kanlow N1, Summer) and 14 progeny switchgrass (*Panicum virgatum* L.) plant clones selected for a diversity of plant form and ranges in lignin levels were tested for leaf resistance to feeding by the fall armyworm (*Spodoptera frugiperda* J. E. Smith), a grass feeding insect pest. Although lignin generally appeared important as a resistance mechanism only at early season stages, replicate clones of two low lignin progeny plants generally remained resistant to fall armyworm feeding. Mechanical damaging increased resistance to fall armyworm feeding in several of these plants. Degrees of resistance were sometimes associated with leaf form of progeny. These results indicate there are likely multiple insect resistance mechanisms operating at different stages in switchgrass, and that segregation of some mechanisms appears related to growth form of the plants.

Keywords Cultivar Kanlow · Culitivar Summer · Ecotypes · Fall armyworm · Full-sib progeny · Insect resistance · *Panicum virgatum* · Sustainable production

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Introduction

Switchgrass (*Panicum virgatum* L.) is a C-4 perennial grass native to tall-grass prairies of the USA. It is an outcrossing species with limited selfing, and can exist in multiple ploidy forms up to octaploid (Vogel et al. 2011). Currently, switchgrass fields are established through seed and different populations of switchgrass with the same ploidy level can be intermated to produce new cultivars. Past and current uses for switchgrass include planting for erosion control and forage use. More recently, switchgrass has been identified as a promising biofuel crop, and new germplasm with improved yields and quality is being developed for the nascent biofuel sector (Bouton 2007; Parrish and Fike 2009; Vogel et al. 2011).

Satisfactory switchgrass material for bioenergy uses will require focused breeding efforts. Intermating of different populations of the same ploidy level has indicated some specific crosses can result in heterosis for yields (Vogel and Mitchell 2008). However, it is unknown if plants improved for yields and biofuel characteristics could have other genetic changes that adversely impact pest resistance, an important characteristic for sustainable production.

Biochemical conversion of switchgrass biomass into liquid fuels is impeded by lignin content (Dien et al. 2008). Reduction of lignin levels through breeding (Sarath et al. 2011) or by transgenic manipulation has improved the production of ethanol from switchgrass (Fu et al. 2011; Saathoff et al. 2011), although the impact of lowering lignin on insect herbivory, a critical component for sustainable production, has not been fully assessed. It is likely that when large scale plantings of switchgrass are deployed, they could be under significant biotic stresses, including herbivory by insects.

Lignin has been identified as a resistance factor against a number of insect species, including grasshoppers and caterpillars. Studies with low or altered lignin lines of maize referred to as brown midrib (*bm*) indicate these plants can have more stalk breakage when damaged by boring insects (Barriere and Argillier 1993). Under expression of lignin due to altering expression of lignifying peroxidase or enzymes involved in the biosynthesis of lignin precursors resulting in lower lignin levels did not significantly reduce insect resistance in underproducers compared to wild type plants in tobacco (Dowd and Lagrimini 1997) or aspen (Brodeur-Campbell et al. 2006) suggesting other factors were still promoting insect resistance. However, transgenic reduction of ferulate crosslinking in fescue (*Festuca* sp.) has led to increased susceptibility to insects (de Buenafina and Fescemyer 2012). Many plant varieties with reduced lignin still have acceptable agronomic properties (Pederson et al. 2005).

Prior studies with switchgrass have indicated insect control can be important in establishment (McKenna and Wolf 1990) and more recent studies have indicated insect pests can potentially interfere with productivity (Dowd and Johnson 2009; Prasifka et al. 2009, 2010). Reduction in lignin levels may enhance the suitability of switchgrass as a host for herbivorous insects (Nabity et al. 2011). However, an initial study with the grass feeding insect pest fall armyworm indicated that resistance in a low lignin forage line was comparable to other normal lignin varieties (Dowd and Johnson 2009) suggesting that reduction in lignin levels may not necessarily result in increased insect susceptibility. Additionally, a saponin that is found in switchgrass has biological activity against fall armyworms (Dowd et al. 2011) at levels comparable to those found in some leaves (Lee et al. 2009; Prasifka et al. 2011). The present study was designed to acquire foundational knowledge on the relative importance of lignin and other plant-based mechanisms impacting the feeding by a non-specialist insect herbivore, fall armyworm, (*Spodoptera frugiperda* J.E. Smith.), for the eventual development of improved plant genetic resources and towards the future understanding in the evolution of insect-plant interactions in switchgrass. Identification of plant mechanisms impacting insect resistance can be utilized to breed for germplasm with sustainable pest resistance.

Methods

Insects

Fall armyworms were reared on pinto bean (*Phaseolus vulgaris* L.) based diet at 27 $\pm$ 1 °C, 50 $\pm$ 10 % relative humidity and at 14:10 light:dark photoperiod as described previously (Dowd 1988). First instar larvae were used for bioassays.

Plants

Plants were obtained by intermating a Kanlow N1 (Vogel and Mitchell 2008) plant as the female parent and a Summer plant as the male parent in a greenhouse in 2004 using methods described previously (Martinez-Reyna and Vogel 2002). Seeds from such crosses display significant genotypic and phenotypic diversity (Vogel et al. 2011), and represent a reasonable means to assess differences in biotic and abiotic stress responses. Seeds from the female parent were after ripened, cold stratified, and germinated on filter paper (Sarath and Mitchell 2008). Individual seedlings were carefully transferred to cone-tainers (Stewe and Sons, Inc., Tangent, OR) containing a soil mix, and maintained in a greenhouse (Martinez-Reyna and Vogel 2002). Approximately 150 seeds were planted and 138 full-sib plants were obtained from this cross. Full sib plants were transplanted to a field nursery located near

Mead, NE in 2005. Plants were divided to yield clonal replicates and transplanted to a new nursery in 2006 in three full blocks of a randomized complete block pattern. Parent plants were similarly maintained in a separate clonal nursery. Field management was similar to that described earlier for similarly spaced plants (Martinez-Reyna and Vogel 2008). From these 138 progeny plants, we selected the 7 plants that had the highest and the 7 plants that had the lowest stem lignin levels for further study, based on initial lignin determinations (see "Method" below) from late season tillers collected from individual plants in 2006 and 2007. Progeny were also characterized as either being Kanlow-like (taller, thicker steams, broader leaves) or Summer-like (shorter, thinner stems, narrower leaves). Leaves from tillers from each of three clonal replicates from each of these 14 lines and parent plants were collected for insect resistance studies in 3 years: late season, August 2008 (late maturity, hard seed); midseason, early and late July 2009 (preflowering and boot stage, respectively); and early season, early May 2010 (spring green up). The flag leaf was collected from each plant in 2008, while in 2009 and 2010 the youngest fully expanded leaf from each plant was removed. The top portion of each leaf was shipped in sealed plastic bags on flaked ice to Peoria.

Bioassays

Leaves shipped overnight on flaked wet ice were used in bioassays. Bioassays were performed in tight fitting Petri dishes as described previously, using ten first instar fall armyworm larvae per dish (Dowd and Johnson 2009). Sections of leaves were taken the same distance from the tips in order to obtain as equal in age/maturity leaf portions across the different lines as possible. Leaves were evaluated visually for feeding damage based on the number of 0.25 mm^2 hole equivalents (approximate head capsule width) consumed over a 2 day period, plus any mortality that was observed, as described previously (Dowd and Lagrimini 1997; Dowd et al. 2007).

Lignin level determinations

For flag leaves collected from each replicate plant in 2008, lignin levels were based on whole tiller samples for only the progeny plants. In 2009 (sample time 2) and 2010, tillers were also harvested at sampling from each parent and progeny plant. Tillers were dried, and leaves were separated from dried tillers for leaf lignin determinations. Lignin levels [as acid detergent lignin (-ADL)] were predicted by near infrared reflectance spectroscopy (NIRS) (Shenk and Westerhaus 1991) using a robust NIRS prediction equation for ADL based on numerous switchgrass laboratory ADL analyses (Vogel et al. 2011). Fiber bag analysis procedures (Vogel et al. 1999) and the ANKOM ADL procedure (ANKOM Technology Corp., Fairport, NY) were used to determine ADL concentration of the switchgrass calibration samples. Before either wet laboratory or NIRS analyses, all harvested samples were dried in a forced-air oven at 50 °C. Dried samples were ground to pass a 2 mm screen in a Wiley mill and a 1 mm screen in a cyclone mill (Thomas-Wiley Mill Co., Philadelphia, PA). For NIRS analyses, the samples were scanned on a near-infrared reflectance spectrophotometer (NIRS Model 6500, Silver Spring, MD). Laboratory means for ADL were used to develop calibration equations by partial least squares (Shenk and Westerhaus 1991). Values for ADL were predicted with a single calibration equation. Calibration statistics including the standard error prediction (SEP) and the R^2 for the prediction equations for each trait were 5.7 g/kg and 0.94, respectively.

Statistical analyses

Statistical differences in mortality were determined by Chi square analysis. Statistical differences in feeding damage and lignin levels were determined by Dunnet's multiple comparison procedure. Correlation analyses were used to determine significant relationships between feeding and lignin levels. All statistical procedures used SAS (SAS Institute, Cary NC) with type I error rates controlled at $\alpha = 0.05$.

Results

Late season assays indicated no association between lignin levels and insect damage

The late season flag leaves collected from both Summer and Kanlow N1 parent (3 clonal replicates, hereafter referred to as clones) were generally more resistant to fall armyworm feeding than those from clones of their progeny (Fig. 1). The leaves from
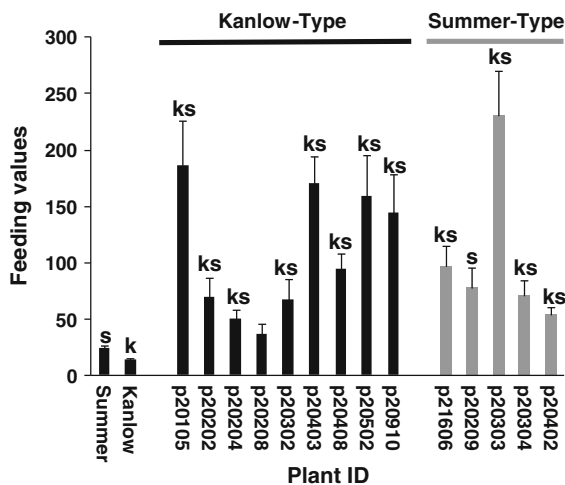
Fig. 1 Fall armyworm feeding damage of late season mature flag leaves for Kanlow N1 × Summer derived switchgrass lines. Feeding values are means ± standard errors of 0.25 mm² hole equivalent totals for 20 leaves from two different plants. *Bars* with a "k" or "s" are significantly different from Kanlow N1 or Summer, respectively, at $P < 0.05$ by analysis of variance. Data from plants phenotypically similar to the female (Kanlow-type, *black bars*) or male (Summer-type, *grey bars*) are grouped. Individual progeny lines are indicated with a "p" in the *x*-axis

clones of progeny plants 20105, 20403, 20502 (all Kanlow-like) and 20303 (Summer-like) were the most heavily damaged, while clones of 20204, 20208 (both Kanlow-like), and 20402 (Summer-like) had the least damaged leaves. Late season stem lignin content ranged from 4.1 to 6.8 (% ADL), and was highly correlated (R = 0.93, $P < 0.0001$) with lignin values for stems of plants grown in the field in 2007. However, there were no significant (at $P < 0.05$) correlations between lignin content and insect feeding (total R = −0.09, Kanlow-like progeny clones R = −0.23, Summer-like progeny clones R = 0.06). Because there was a slight trend for the Kanlow-like progeny clone resistance to be associated with lignin levels, lignin determinations were continued for the leaves assayed for insect resistance in the subsequent years. Examining the progeny clone plants individually compared to parent clone plants did suggest factors other than lignin were also important in insect resistance. For example, Kanlow-like progeny clones with high levels of lignin (20105, 20502, 20910) had leaf feeding ratings significantly higher than either parent, and yet were in the same range as Kanlow-like progeny clones of plant 20403, which had low lignin.

Midseason progeny leaves were generally more resistant to insects than parent leaves

In contrast to results noted with late season flag leaves, the actively growing mid season leaves collected from several of the progeny plant clones often were more resistant to fall armyworms than those from Summer or Kanlow N1 parent clones, suggesting a heterotic improvement in insect resistance over the parents at this stage (Fig. 2). Leaves from clones of 20502 and 20910 (both Kanlow-like) were among the most heavily damaged, and leaves from clones of 20302, 20403 (both Kanlow-like), 20402 and 21606 (both Summer-like) were among the least damaged. Leaves from clones of 20303 (Summer-like) were among the most highly damaged, as they were in 2008. The leaves from clones of two Summer-like progeny plants previously mentioned had ca. 11X and 8X less damage than leaves of clones of the Kanlow N1 parent (Fig. 2a). Feeding rates were generally unchanged or significantly decreased (clones of Kanlow N1, clones of Kanlow-like progeny plants 20105, 20204, 20502, 20910) from the first to the second mid season leaf collection, although feeding significantly increased from the first to the second leaf collection for clones of Summer, and clones of the Kanlow-like progeny line 20403 (Fig. 2b). There were no significant correlations between lignin levels and feeding damage on the leaves overall or for clones of Kanlow-like or Summer-like progeny examined individually, for leaves from either damaged or undamaged plants.

Some association was noted between early season leaf damage and lignin levels

Fall armyworm feeding damage of early season progeny plant clone leaves generally was similar to that of clones of Summer and Kanlow N1 parents (Fig. 3). The leaves from clones of the Summer parent often were damaged to a greater extent than those from clones of the Kanlow N1 parent, which was the same trend noted for flag leaves. However, there were exceptions, as leaves from clones of the Kanlow-like progeny 20202 had significantly less damage compared to leaves from clones of Kanlow N1, and leaves from clones of several progeny had significantly less damage than leaves from clones of Summer, including clones of the Summer-like progeny 20209. Leaves from the Kanlow-like progeny 20408 the Summer-like
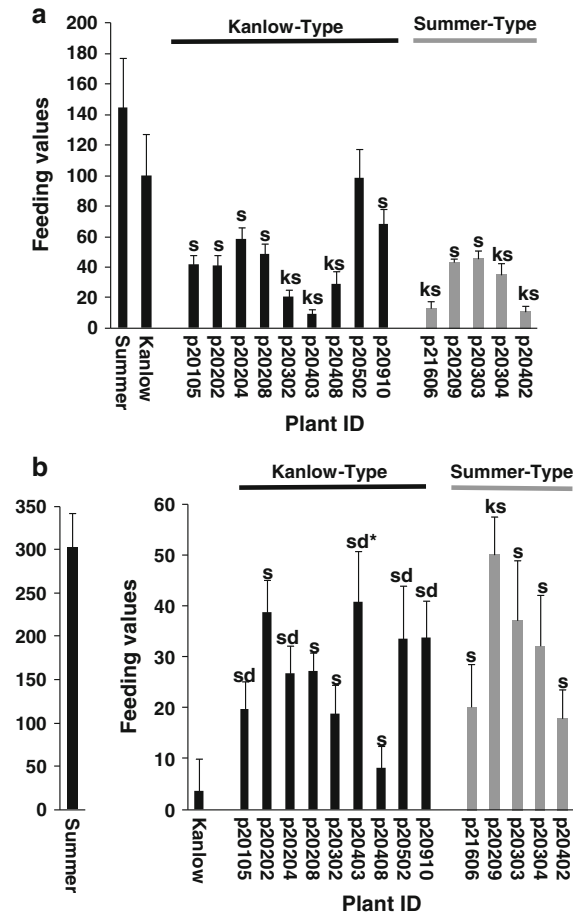
Fig. 2 Fall armyworm feeding damage of uppermost fully expanded leaves collected from plants midseason at late vegetative (a) and boot stage (b). Feeding values are means ± standard errors of 0.25 mm² hole equivalent totals for 9 leaves from tillers of three different plants. Bars with a "k" or "s" are significantly different from Kanlow N1 or Summer, respectively, at $P < 0.05$ by analysis of variance. Bars "boot stage" with a "d" are significantly different from values observed for leaf samples harvested at the late vegetative stage at $P < 0.05$ by analysis of variance. *Only Kanlow-like progeny where damage ratings increased with plant age. Plants phenotypically similar to the female (Kanlow-type, black bars) or male (Summer-type, grey bars) are grouped. Individual progeny lines are indicated with a "p" in the x-axis
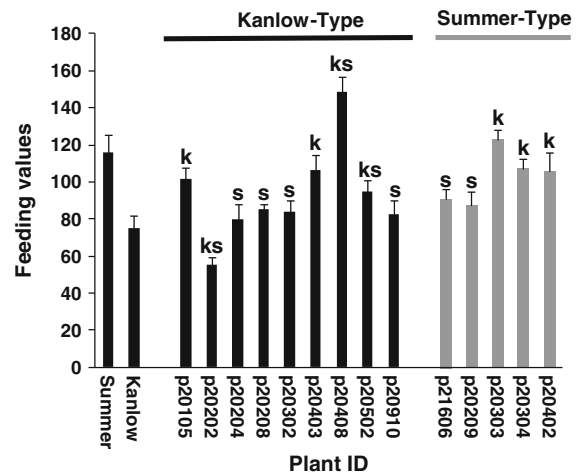
Fig. 3 Fall armyworm feeding damage of early season uppermost emerging leaves collected from plants at early vegetative stage. Feeding values are means + standard errors of 0.25 mm² hole equivalent totals for 9 emerging leaves from tillers of three different plants. Bars with a "k" or "s" are significantly different from Kanlow N1 or Summer parent, respectively, at $P < 0.05$ by analysis of variance. Plants phenotypically similar to the female (Kanlow-type, black bars) or male (Summer-type, grey bars) are grouped. Individual progeny lines are indicated with a "p" in the x-axis

progeny 20303 were among the most damaged in early season. Leaves from clones of both of these lines were more resistant than leaves from clones of the parents when tested at mid season (compare Figs. 2, 3). Feeding and lignin levels were significantly correlated overall for the early season leaves (R = −0.79, $P = 0.0008$), which mainly appeared due to clones of Kanlow-like plants (R = −0.86, $P = 0.0028$), as

correlations for clones of Summer-like plants were not significant (R = −0.67, $P = 0.21$). The most resistant progeny, 20202, also had the highest lignin level.

Except for the 20.5 % mortality of fall armyworm larvae fed late season leaves from Kanlow N1 clones, substantial larval mortality was only noted for the midseason leaves assayed. The midseason leaf mortality was most pronounced for larvae fed leaves from the second collection (Table 1). At least one leaf replicate caused more than 20 % mortality of fall armyworm larvae, and some (clones of Kanlow-like 20302 and 20408 and Summer-like 20402 and 21606) had more than 40 % of leaves assayed that caused greater than 20 % mortality in the first leaf collection. Mortality was generally greater for the second leaf collection, increasing to over 50 % for all clones but those of Summer. The number of clones of progeny with leaves causing over 75 % mortality of fall armyworm larvae was more widespread as well, and again included clones of 20302, 20402, 21606, but now also included clones of Kanlow-like 20105, 20204, 20208, 20302; and clones of Summer-like 20209 and 20303, which had more than 50 % of replicates with greater than 75 % mortality.

Overall rankings indicated some progeny had low lignin and good insect resistance

Relative rankings were fairly consistent over the three seasons and thus an average is useful for elucidating plant resistance and lignin relationships (Fig. 4). The Summer parent plant had consistently higher insect damage and was somewhat lower in lignin content. In contrast, the Kanlow parent showed slightly higher lignin content (lower ranking) and displayed greater resistance to insect feeding. Leaves from clones of Kanlow-like progeny lines 20202, 20204, 20208, 20302 and clones of Summer-like 20402 and 21606 had among the least damage overall, while leaves from clones of Summer-like 20303 and Kanlow-like 20502 and 20105 had the most damage overall (Fig. 4). Of the least damaged examples, leaves from clones of 20402 and 21606 had relatively low lignin levels, whereas leaves from clones of 20202 and 20302 had

relatively high lignin levels. Interestingly, clones of 20402 and 21606 also had the highest frequency of assay dishes with more than 20 % mortality for the growing plant leaves from the first collection in midseason. For the most damaged examples, leaves from clones of Kanlow-like line 20408 had a relatively low lignin level, while leaves from clones of Kanlow-like line 20502 had a relatively high lignin level. Overall, lignin levels were more consistent between mid season and early season leaves (correlation R = 0.99, P < 0.0001), but the same trends were generally found for comparisons with late season tillers as well (R = 0.82, P = 0.0003 for midseason and R = 0.84 and P = 0.0002 for early season). Statistical analysis of the multiple samples taken in mid season from each line indicated four examples (clones of Summer-like 20303, 20304, 20402; and Kanlow-like 20408) had significantly lower levels of lignin than clones of Kanlow N1.

Table 1 Day 2 percent mortality levels of fall armyworm larvae fed switchgrass leaves in 2008, and 2009 assays

	2008	2009-1 % leaves		2009-2 % leaves		Type[a]
		Total	>20 %	Total	>75 %	
Parents						
Summer	8.0	6.1	0.0	15.4k	0.0	S
KanlowN1	20.5	13.6	11.1	62.2sd	22.2	K
Progeny						
20105	1.0	5.5	11.1	81.5ksd	77.8	K
20202	3.5	9.2	11.1	70.0sd	44.4	K
20204	5.0	4.2	0.0	88.0ksd	66.7	K
20208	9.0	7.5	11.1	92.0ksd	77.8	K
20209	4.5	9.9	11.1	83.0ksd	77.8	S
20302	5.5	28.1ks	44.4	83.7ksd	55.6	K
20303	0.0	7.2	11.1	79.2ksd	55.6	S
20304	2.5	8.3	33.3	63.3sd	33.3	S
20402	0.5	37.5ks	66.7	77.1sd	66.7	S
20403	0.0	21.8 s	22.2	58.2sd	11.1	K
20408	0.0	22.8 s	44.4	74.1sd	33.3	K
20502	1.0	9.4	22.2	57.9sd	33.3	K
20910	6.5	12.1	33.3	57.1sd	33.3	K
21606	0.5	43.9ks	77.8	80.4ksd	55.6	S

Mortality values are totals for 20 leaves from tiller progeny from two different plants (2008) and 9 leaves from tiller progeny of three different plants (2009). Values in columns followed by a "k" or "s" are significantly different from Kanlow or Summer, respectively, at P < 0.05 by Chi square analysis. Values in the 2009-2 column followed by a "d" are significantly different from early 2009 plant values at P < 0.05 by Chi square analysis. Summer was the male parent and KanlowN1 was the female parent

[a] Type = Phenotypic similarity (K = Kanlow-like; S = Summer-like)

Discussion

Insect resistance in switchgrass leaves is developmentally influenced and inducible

In this first study of insect resistance mechanism genetics in switchgrass, we have shown that multiple insect resistance mechanisms appear to be operational in genetically-related full-sib family of switchgrass plants, derived from a cross between an upland and a lowland tetraploid cultivar. These progeny plants displayed a range of phenotypes and could be classified (based on plant form) as more similar to the male (cv. Summer) or female (cv. Kanlow) parent. These phenotypic differences would indicate diversity within these plants at the genetic level, and suggest that such full and half-sib families of switchgrass could serve as genetic resources to evaluate the evolution of insect resistance mechanisms within this important bioenergy species.

Significant differences in resistance to fall armyworm feeding have been reported for several commercial switchgrass lines, and relative resistance can vary by plant maturity (Dowd and Johnson 2009). In the present study, we also noted differences in resistance of several progeny clones compared to
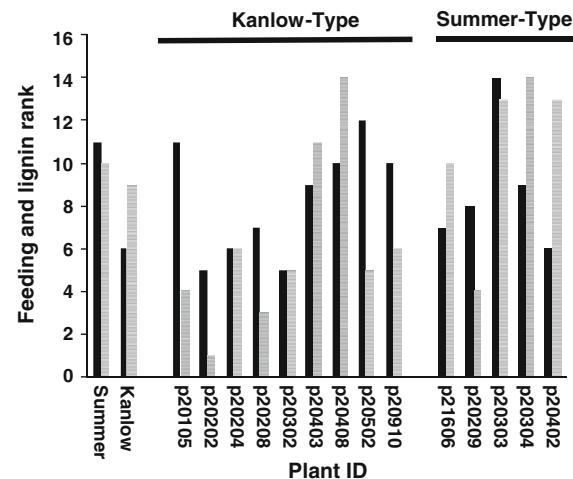

Fig. 4 Relative insect resistance rank and lignin level rank in the parent and progeny switchgrass plants averaged over 3 years. *Black bars* are insect resistance rank and *grey stripped bars* are lignin levels. Plants phenotypically similar to the female (Kanlow-type, *black bars*) or male (Summer-type, *grey bars*) are grouped. Individual progeny lines are indicated with a "p" in the *x*-axis

parents, depending on the stage examined, suggesting insect resistance is genetically complex. Levels of insect resistance chemicals in leaves of grasses such as maize can change over the life of the plants (Cambier et al. 2000). Developmental changes also result in the reduction in expression of several putative insect resistance genes in maize as the plant develops (Johnson et al. 2011), so many possibilities exist for the resistance mechanisms that are subject to change.

Additionally, the increase in fall armyworm mortality noted in the present study between the two midseason leaf collections or several switchgrass progeny clones suggests wounding strongly induced insect resistance mechanisms. Mechanical wounding has induced resistance to aphids (*Rhopalosiphon padi* L.) in maize (*Zea mays* L.) (Morse et al. 1991), and insect feeding has induced resistance to caterpillars in tobacco (*Nicotiana tabacum* L.) and tomato (*Solanum lycopersicum* L.) (Dowd and Lagrimini 1997; Dowd et al. 1998). Insect feeding can also differentially induce insect resistance mechanisms in maize (McMullen et al. 2009), so gene expression studies of this type would be valuable in determining if the same genes are up or down regulated in switchgrass as an indication of how domestication may influence insect resistance gene expression.

Lignin is one of several insect resistance mechanisms of switchgrass

Lignin content, peroxidases and associated traits appear to be important for plant resistance towards insect herbivory (Dowd 1994; Heng-Moss et al. 2004; Garcia-Lara et al. 2007; Gutsche et al. 2009). For the switchgrass parent and progeny plants evaluated in this study, lignin content of leaves appeared to have a modest effect on insect resistance, and the role(s) of peroxidases in this process remains to be investigated in the future. Insect resistance has been generally associated with increased levels of peroxidase expression, and insect resistance has been increased in studies using transgenic means to increase peroxidase levels (Dowd et al. 2008, 2010), although mechanisms other than lignification can be involved in peroxidase-based resistance (Dowd et al. 2008). Differential expression of peroxidases could be among the factors impacting the variation in leaf damage observed in our study, as differences in peroxidase isozyme activity have been reported for commercial switchgrass

cultivars with different levels of insect resistance (Dowd and Johnson 2009). However, in the present study mature switchgrass leaves that retained insect resistance did not necessarily have high lignin levels (compare clones of 20105 and 20208, both high lignin). It is possible that below a certain threshold of other co-occurring resistance molecules, lignin becomes a relatively more important insect resistance factor. Factors other than lignin are apparently contributing to insect resistance in clones of low lignin plants 20402 and 21606, and for the other progeny clones during mid and late season. Switchgrass has pest resistance genes homologous to those from maize, and variations between the sequence of four representative enzymatic resistance genes have been reported for several switchgrass lines (Dowd and Johnson 2009).Maize and switchgrass are likely to have similar chemical resistance factors, and there is indirect evidence that switchgrass produces the common resistance factor DIMBOA found in maize and wheat (Lin et al. 2008). Conversely, maize and many other cultivated grains do not produce saponins (Papadopoulou et al. 1999), which act as insect resistance molecules in a number of other plant species (DeGeyter et al. 2007). The saponin protodioscin, produced by switchgrass (Lee et al. 2009) has been identified as a resistance factor against corn earworms (*Helicoverpa zea* Boddie.) and fall armyworms (Dowd et al. 2011) at naturally occurring levels. Older leaves of Kanlow with high levels of protodioscin were more resistant to the armyworm (Prasifka et al. 2011). The high mortality of fall armyworms when fed midseason leaves from clones of the two low lignin plants (20402 and 21606) also suggests that toxic factors other than lignin are present.

Additionally, the increased feeding rates, coupled with increased mortality levels noted between the first and second midseason leaf collection for clones of plant 20403, and increased mortality on leaves from plants 20202, 20208, 20209, 20303, 20304, suggest that factors interfering with nutrition are involved. Dilution of nutrients with nondigestible compounds such as cellulose can trigger compensatory feeding (Lee et al. 2004) including in the fall armyworm (Wheeler and Slansky 1991). Thus, factors other than lignin that could be interfering with nutrition are likely to be influencing resistance levels in this stage of plant development.

Switchgrass leaf resistance to insects is associated with plant growth form

There was some evidence that insect resistance was associated with growth form (Summer-like or Kanlow-like) and that both negative and positive heterotic interactions were occurring that affected resistance compared to levels seen for either parent a particular stage. For the early season leaves, most Kanlow-like leaves were damaged to a significantly higher extent than the Kanlow parent, while most Summer-like leaves were damaged to lower extent than the Summer-parent. Low lignin levels tended to be more associated with Summer-like than Kanlow-like growth form, although exceptions did occur, indicating this trait did have some independence from form. Leaves from clones of more Kanlow-like than Summer-like progeny had resistance significantly induced for the second compared to the first leaf sampling in midseason, which did not appear lignin-related. Enhanced insect resistance of tomato expressing high lignin peroxidase activity was associated with a smaller fruit size (Dowd et al. 1998).

Some full-sib progeny of tetraploid switchgrasses display a useful assortment of insect resistance mechanisms

Polyploidy is important in plant evolution and has been used to generate novel phenotypic properties for crop plants (Nuismer and Thompson 2001). Overall our results indicate that significant changes in lignin levels can be observed in some of the progeny obtained by mating a tetraploid forage-type Summer plant with a lowland-type Kanlow plant. Yet, some of the low lignin progeny still maintain resistance to the fall armyworm, which include the more productive Kanlow-like growth form. Tests with other potential switchgrass insect pests are needed to determine how broad spectrum this result might be, as well as tests against reported switchgrass pathogens (Zeiders 1984; Gravert et al. 2003; Gustafson et al. 2003). It is possible that disease resistance will parallel insect resistance as many of the same mechanisms are involved (Dowd et al. 2008; Johnson et al. 2008). Hybrid versions of switchgrass, similar to the progeny lines tested in the current study, can have much higher yields than their parents (Pederson et al. 2005; Vogel and Mitchell 2008), and could possess enhanced insect

resistance (this study). Together, these data suggest that reduction in lignin levels may not adversely affect yield or other production factors, providing there is sufficient pest resistance conferred through other heritable mechanisms. Once the mechanisms are identified and understood, this information can be used to assist in developing sustainable lines of switchgrass for biomass production.

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