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Impact of Reduced Lignin on Plant Fitness

J. F. Pedersen,* K. P. Vogel, and D. L. Funnell

ABSTRACT

Lignin content of crop plants has been reduced by traditional plant breeding, natural and induced mutations, and insertion of transgenes. The effects of these genes and associated lower lignin content have been examined in terms of agricultural fitness or with regard to economically harvestable yields of useful plant products, or, in the case of some perennial species, survivability over multiple years. In general, crop yields are depressed by significant reductions in lignin content. Other negative effects observed in plants with lowered lignin contents include lodging and reduction of long-term survival of some perennial species. However, the interactions of genes involved in lignin metabolism with genetic background and the environment in which the low-lignin crop is cultivated are substantial. Examples are provided that demonstrate that lignin can be reduced in specific lines or populations without damaging fitness. It is concluded that it will be essential to incorporate lignin reducing genes into numerous genetic backgrounds and combinations, and evaluate the resulting lines in diverse environments, to discover optimal combinations and to obtain a true measure of value and fitness in agricultural systems.

FROM A CLASSIC ECOLOGICAL OR evolutionary viewpoint, fitness is defined as the relative reproductive success of a genotype as measured by survival, fecundity, or other life history parameters (Schlindwien, 2002). But, as stated by Valladares (1999) “although Darwinian fitness is conceptually straightforward, it is extraordinarily difficult to measure directly” and “can be measured only in relative terms, and usually through indirect parameters such as longevity, size, or growth which are frequently correlated with Darwinian fitness.” Falconer (1989) further complicates the concept by reminding us that “...fitness of a genotype with respect to any particular locus is not necessarily the same in all individuals. It depends on the environmental circumstances in which the individual lives, and also on the genotype with respect to genes at other loci.”

From an agricultural viewpoint, Darwinian fitness is often not relevant because artificial selection is practiced. Agronomically favorable traits are not necessarily those that would promote survival of wild species in at least some environments. Life-spans are modified to accommodate growing seasons. Progeny are harvested

and utilized before maturity or dissemination. Fecundity or clonal reproduction is controlled. Agriculturally fit plants are those that are the most useful to humans in agricultural systems, and their fitness must be measured with regard to such systems.

In annual cropping systems, agricultural fitness is therefore defined as the ability to produce economically harvestable yields of useful and commercially desirable plant products. In perennial cropping systems, agricultural fitness is defined as the ability to produce economic yields of useful plant product across multiple years. Like the metric components of fitness described by Falconer (1989), agricultural fitness is influenced by many metric factors, including germination, pest resistance, survival, growth rate, lodging, and yield. We will examine the effect of reduced lignin on such measurable components of agricultural fitness. Because of the strong interaction of environment on fitness defined in these terms, we will largely limit our discussion to data derived from the field.

Before examining the effects of reduced lignin on plant fitness, a brief review of lignin functions in whole plants is useful. Walter (1992) simply states “lignin is one of the prerequisites for terrestrial plant life.” Campbell and Sederoff (1996) state “lignin, a complex phenolic polymer, is important for mechanical support, water transport, and defense in vascular plants.” Lignin provides strength and rigidity to plants and to plant structures such as xylem, which allows for the transport of water; and sclerenchyma and bundle sheath cells, which provide a natural barrier to microorganisms (Bird, 1988). Production of lignin has been demonstrated to be induced in plants in response to mechanical damage or infection by pathogens in numerous species including cucumber (*Cucumis sativus* L.), carrot [*Dacus carota* subsp. *sativus* (Hoffm.) Arcang], narcissus (*Narcissus peoticus* L.), radish (*Raphanus sativus* L.), melon (*Cucumis melo* L.), hop (*Humulus lupulus* L.), potato (*Solanum tuberosum* L.), bean (*Phaseolus* spp.), and grasses (Bird, 1988).

Lignin is integral to plant health, survival, and functions. In classical Darwinian fitness terms, the answer to the question “Does reduced lignin impact plant fitness?” is very probably “Yes,” especially with respect to natural populations. For example, glume and caryopses lignin content of the wild relative of sorghum, shattercane [*Sorghum bicolor* subsp. *drummondii* (Nees ex Steud.) de Wet & Harlan], is higher than that of cultivated sorghum [*Sorghum bicolor* (L.) Moench], and lignin content is positively correlated with over winter survival of shattercane seed (Fellows and Roeth, 1992). However, in terms of agronomic fitness, reducing lignin may have positive, neutral, or negative effects. In the following pages, we will explore these possible effects in various crop species.

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Multiple Sources of Reduced Lignin and Implications

Returning to the quote from Falconer (1989) "The fitness of a genotype with respect to any particular locus is not necessarily the same in all individuals. It depends on the environmental circumstances in which the individual lives, and also on the genotype with respect to genes at other loci," it is important to recognize that numerous transgenic events, induced mutations, and naturally occurring mutations resulting in reduced lignin in numerous species contribute to the current body of knowledge. A summary of plant species, modified genes, and effects on lignin content (0–95% reduction) and composition is provided by Dixon et al. (2001). As will be shown in the following discussions, variable conclusions regarding the effect of reduced lignin on agricultural fitness are common and most probably derive from interactions of specific genes (which may affect more than just lignin synthesis) and individual plant species, cultivars, and environments.

Maize

Several authors provide insights into the relationships of lignin content and agricultural performance without the use of mutations or transgenes. Following five cycles of selection for high and low stalk crushing strength, Undersander et al. (1977) showed no change in lignin content in maize (*Zea mays* L.) stalks derived from either of two source populations and concluded that stalk strength and lodging resistance can be increased with little effect on stalk composition. In three populations based on individuals selected for extreme divergence in fiber concentration, Wolf et al. (1993) showed only weak and inconsistent correlations between lignin content and various agricultural fitness parameters (height, grain yield, total yield, lodging, days to silk). Evaluation of lignin content in two maize hybrids resistant to fall armyworm (*Spodoptera frugiperda* J.E. Smith) and southwestern corn borer (*Diatraea grandiosella* Dyar) and two maize hybrids susceptible to these insects showed no difference in lignin content due to hybrid (Williams et al., 1998) in nonchallenged plants. It appears that in normal maize, naturally occurring variation in lignin content has little discernable effect on agricultural fitness.

Brown Midrib

Four brown midrib mutations are known in maize (*bm1*, *bm2*, *bm3*, and *bm4*) and are associated with significant alterations in lignin composition and reductions in lignin content in the stover. The scientific literature contains considerable information about the effects of these genes, especially *bm3*, on plant composition and on agricultural fitness. The *bm3* mutation results in reduced caffeic acid *O*-methyltransferase (COMT) activity (Vignols et al., 1995), while the *bm1* mutation reduces cinnamyl alcohol dehydrogenase (CAD) activity (Haplin et al., 1998). The reader is cautioned that the following is a discussion of the effects on fitness parameters of maize in lines containing these lignin reducing genes, not a

discussion of the direct effects of reduced lignin on agricultural fitness.

bm3. Miller et al. (1983) developed a set of S_1 lines and $S_1 \times S_1$ hybrids from three populations segregating for *bm3*. Brown midrib lines had lowered grain, stover, and fodder yields (averaged 77, 90, and 84% of normal lines, respectively), a 10% reduction in average seedling dry weight, and a 15% reduction in dry matter accumulation across the growing season (Miller et al., 1983; Miller and Geadelmann, 1983). Brown midrib lines also exhibited higher incidence of stalk breakage at maturity, 2- to 4-d-later maturity, and reduced vigor during vegetative growth as compared with normal lines (Miller et al., 1983). There was considerable genetic variation among the *bm3* lines, but the authors concluded that although early vigor and growth rates could be improved to acceptable levels through breeding, improvement of *bm3* line grain yields to that of normal maize lines was unlikely (Miller and Geadelmann, 1983).

Using a set of 15 *bm3* lines and their 15 normal isogenic lines, Lee and Brewbaker (1984) showed average grain yields of the brown midrib lines to be reduced by 20%, and average stover yields to be reduced by 17%. Average reductions associated with *bm3* for kernel number per row (12%), filled ear length (10%), ear height (7%), plant height (5%), and stem diameter (5%) were also documented (Lee and Brewbaker, 1984). Again, considerable variation among *bm3* lines was noted, but the authors implied that it is unlikely that these reductions in fitness could be overcome by breeding. They also excluded linkage of the *bm3* gene and yield-reducing genes as the mechanism for yield reduction and intimate pleiotropism and reduced photosynthesis.

Reduction in dry matter yield (15–20%) in *bm3* isolines compared with their normal counterparts has been reported by Inoue and Kasuga (1989). Gentinetta et al. (1990) demonstrated similar yield reductions due to *bm3* in 21 hybrids compared with their isolines counterparts but indicated that yield reduction was genotype specific and in one genetic background, the *bm3* line had virtually identical yield to its normal counterpart. Lines developed by Weller et al. (1985) did not show yield differences between *bm3* and normal isolines, but silking was delayed an average of 3 d in the *bm3* isolines. Although a specific brown midrib gene is not identified, Cox and Cherney (2001) confirmed a 20% reduction in yield in commercial brown midrib hybrids when compared with other commercial hybrids, and noted a reduction in early season growth in the brown midrib hybrids.

Increased lodging is generally assumed to be associated with brown midrib maize. However, an increase in lodging attributable to brown midrib was not detected in several studies, possibly because of overriding effects of genetic backgrounds (Weller et al., 1985; Inoue and Kasuga, 1989), maturity (Miller et al., 1983), or environmental or other experimental design factors. However, in a study designed specifically to evaluate stalk strength, Zuber et al. (1977) showed a 17 to 26% decrease in crushing strength in three *bm3* hybrids compared with their normal counterparts.

bm1, bm2, bm4. Although cell wall composition in all four maize brown midrib mutants has now been described (Marita et al., 2003), little has been published describing the effects of *bm1*, *bm2*, and *bm4* on the agricultural fitness of maize. The *bm1* mutation appears to decrease days to flowering while the *bm2* mutation appears to increase days to flowering (Vermerris and McIntyre, 1999). Vermerris et al. (2002a) later demonstrated that *Bm1/bm1* maize plants flower earlier than their homozygous counterparts. The authors speculate that changes in lignin composition may mimic drought stress and alter plant development, but conclude that there is no evidence that the *bm1* gene (heterozygous or homozygous) mimics drought stress or affects early growth and development of maize (Vermerris et al., 2002a). A single study reports no reduction in dry matter yields in *bm1* maize hybrids (Barriere et al., 1994).

Transformation

Reduction in lignin content of maize has recently been accomplished by downregulation of OMT (He et al., 2003) and COMT (Piquemal et al., 2002). Both studies utilized individual greenhouse grown plants. He et al. (2003) reported no detectible differences in dry matter yield due to reduced OMT because of highly variable yield data. Piquemal et al. (2002) did not report yield data but indicate that the effect of downregulating COMT on agronomically important traits is currently under investigation.

In conclusion, other than minor shifts in days to flower (1–2 d), only the *bm3* mutant of maize has been demonstrated to have appreciable effects on agricultural fitness. These effects are often genotype dependent, but the reduction in yield associated with the *bm3* mutant is so significant that when combined with other negative effects it will be difficult to produce maize *bm3* hybrids equivalent to normal hybrids in terms of agricultural fitness. Sources of reduced lignin in maize and their effects on agricultural fitness are summarized in Table 1.

Sorghum

Although lignin chemistry and cell wall composition has been extensively reported in the literature, very little information is available on the effects of lignin

Table 1. Effects of reduced lignin on agricultural fitness of maize.

Source of variation	Effect on agricultural fitness
Divergent selection for fiber concentration	weak and inconsistent correlations between lignin content and height, yield, lodging, and maturity
Insect resistant and susceptible hybrids	no difference in lignin content
<i>bm3</i> (reduced COMT activity)	reduced grain yield reduced dry matter yield reduced early season vigor 2- to 4-d-later maturity decreased stalk strength increased stalk breakage
<i>bm1</i> (reduced CAD activity)	decreased days to flowering no reduction in dry matter yield no effect on early season growth increased days to flowering
<i>bm2</i> <i>bm4</i>	no information

content on agricultural fitness. We will briefly summarize the different traits that have been the foci of various studies.

Insect Resistance

A study was conducted, comparing six lines moderately resistant to stemborer [*Chilo partellus* (Swinhoe)] and shootfly [*Atherigona soccata* (Rond.)], and three lines susceptible to these insects (Khurana and Verma, 1983). No relationship between lignin and insect resistance was observed in plants 30 d of age. Although the group of resistant lines had slightly lower average lignin content than the group of susceptible lines (6.3 and 7.3%, respectively) at 50 d of age, the results were inconclusive due to a wide range in lignin content among the resistant lines (5.0% to 8.2%).

In a study examining 42 sorghum conversion¹ lines for chemical makeup (crude protein, neutral detergent fiber, tannin, and lignin content) and resistance to the fall armyworm [*Spodoptera frugiperda* (J. E. Smith)], Moussa et al. (1991) observed significantly higher average lignin content in panicles of lines identified as resistant (7.9% lignin) than lines identified as susceptible (5.9% lignin). However, within each resistant or susceptible group of sorghum lines, there was considerable variation in lignin content with susceptible lines ranging from 2.7 to 8.7% lignin, and resistant lines ranging from 3.4 to 13.9% lignin (Moussa et al., 1991). Within the resistant group, lignin concentration was negatively correlated (strength of correlation not reported) with duration of larval stage (Moussa et al., 1991). These seemingly contradictory results lead us to conclude that a relationship between reduced lignin content and susceptibility to insects has yet to be clearly demonstrated.

Brown Midrib

Brown midrib mutants of sorghum were first described by Porter et al. (1978) and are associated with significantly reduced lignin content in affected plants. Three of those mutations, *bmr-6*, *bmr-12*, and *bmr-18*, have been the focus of considerable study on lignin chemistry, cell wall composition, and animal performance, but surprisingly little information is available on their impact on agronomic fitness. The genes *bmr-12* and *bmr-18* are allelic (Bittinger et al., 1981) but not identical (Bout and Vermerris, 2003). In some instances—especially in extension and popular literature—brown midrib is used to describe a phenotype, and specific mutations are not identified. It has been suggested that “degree of coloration has no meaning” (Butler, 2003). However, in our laboratory, greenhouse, and field studies, we consistently observe earlier expression and more intense coloration in *bmr-6* lines than in *bmr-12* lines (data not shown). These mutations are quite different in their effect on lignin chemistry. The

¹A backcrossing program in which tropically adapted sorghum accessions are converted to lines suitable in height for harvest by commercial combines and that will reach maturity in temperate latitudes (Stephens et al., 1967).

bmr-6 mutation exhibits reduced CAD activity (Pillone et al., 1991), while the *bmr-12* and *bmr-18* mutations exhibit reduced COMT activity (Bout and Vermerris, 2003).

Extension and popular literature give strong indication that brown midrib is associated with lodging in forage sorghum. Our own experience and knowledge of producer experience with brown midrib forage sorghum is in complete agreement.

***bmr-6*.** Although it is generally believed that the effect of sorghum brown midrib mutations on yield is similar to those reported in maize (Kalton., 1988), only one report of yield differences in normal and brown-midrib counterparts of sorghum has been published. In a multi-state forage trial comparing normal and *brm-6* 'Piper' and normal and *brm-6* 'Greenleaf' sudangrass [*Sorghum bicolor* subsp. *drummondii* (Nees ex Steud.) de Wet & Harlan], Casler et al. (2003) found the brown midrib phenotype as a result of *bmr-6* to be environmentally and cultivar sensitive. First harvest yields of *bmr-6* Greenleaf were reduced in Wisconsin (15%), but not in Nebraska, while yields of *bmr-6* Piper were reduced in both Wisconsin and Nebraska (32 and 27%, respectively) as compared with their normal counterparts (Casler et al., 2003). Reduced tillering, and reduction in plant height were observed in the *bmr-6* lines across environments (Casler et al., 2003). Second harvest yields of *bmr-6* lines were also reduced across environments and varieties, indicating a limitation in re-growth potential in *bmr-6* sorghum (Casler et al., 2003).

Unlike the *bm1* CAD mutation of maize which reduces days to flowering by approximately 2 d, the *bmr-6* mutation in sorghum has been shown to delay flowering by 5 d when compared with wild-type controls (Vermerris et al., 2002b).

***bmr-12* and *bmr-18*.** Only two reports of the specific effects of *bmr-12* and *bmr-18* on plant agronomic fitness were found in the literature. Vermerris et al. (2002b) noted an average 7-d reduction in days to flower in two *bmr-12* sorghum lines and essentially no change in days to flower in two *bmr-18* sorghum lines compared with their wild-type counterparts. In a study using an F₂ population segregating for various traits, Kasuga et al. (2001) conclude that the *bmr-18* gene has no negative effects on resistance to sheath blight (*Rhizoctonia solani* Kühn).

In conclusion, naturally occurring variation in sorghum lignin content could not be convincingly correlated with resistance or susceptibility to insects. The brown midrib mutations increased lodging, and reduced yield, regrowth, height, and tillering. Effects of the *bmr-6* mutation were shown to be sensitive to both environment and genetic background. The various brown midrib genes affected days to flower by up to 1 wk with *bmr-12* being the most severe. Sources of reduced lignin in sorghum and their effects on agricultural fitness are summarized in Table 2.

Pearl Millet

Brown midrib mutants have been developed in pearl millet (*Pennisetum glaucum* L.) by ethyl methyl sulfo-

Table 2. Effects of reduced lignin on agricultural fitness of sorghum.

Source of variation	Effect on agricultural fitness
Insect resistant and susceptible lines	lignin content and insect susceptibility questionable
Brown midrib (no mutation identified)	increased lodging
<i>bmr-6</i> (reduced CAD activity)	reduced dry matter yield reduced regrowth following harvest reduced height reduced tillering environment and variety sensitive increased days to flower
<i>bmr-12</i> (reduced COMT activity)	decreased days to flower
<i>bmr-18</i> (reduced COMT activity)	no change in days to flower no change in sheath blight susceptibility

nate (Cherney et al., 1988) and through discovery of a naturally occurring mutation (Degenhart et al., 1995). The naturally occurring brown midrib mutation is thought to be similar to the chemically induced mutation and to *bmr-12* in sorghum. Degenhart et al. (1995) demonstrated a 23% reduction in yield in lines with the brown midrib mutation, hypothesized to be due to a block of genes involved in biomass production, linked to the brown midrib gene. The effect of brown midrib on pearl millet yield is shown in Table 3.

Small Grains

Unlike maize and sorghum, variation in structural lignin content and its effect on agricultural fitness has received little attention in wheat (*Triticum aestivum* L.) and other small grains. However, a wealth of information on wound-induced lignin and its role in disease resistance in wheat, oat (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) has been documented. Such induced lignification typically occurs at the wound site and involves enzymes such as CAD (Mitchell et al., 1999). It seems reasonable to hypothesize that genes such as brown midrib that have major effects on structural lignin synthesis would also affect wound induced lignification, but such relationships have not yet been established. Thorough reviews of wound induced lignification and its relationship to disease resistance include Vance et al. (1980), Bird (1988), and Ride et al. (1989), and are not discussed further in this review.

Several studies have attempted to establish a relationship between lodging and lignin content in wheat. Low lignin content was observed in the basal internodes of lodged wheat (Mulder, 1954). However, more recent studies using growth regulators to elicit reduced lodging showed no relationship between lignin content and lodging in wheat (Clark and Fedak, 1977; Knapp et al., 1987), barley (Clark and Fedak, 1977; Stanca et al., 1979) and

Table 3. Effects of reduced lignin on agricultural fitness of other annual crops.

Species and source of variation	Effect on agricultural fitness
Pearl millet	
Brown midrib (naturally occurring mutant)	decreased yield
Wheat, oat, and barley	
Growth regulators and variety	no effect
Tobacco	
Transformation	reduced growth in some instances

oat (Clark and Fedak, 1977). Sources of reduced lignin in small grains and their effects on agricultural fitness are summarized in Table 3.

Tobacco

Because of ease of transformation and the large body of scientific knowledge regarding tobacco (*Nicotiana tabacum* L.), much of the basic research on lignin, including biosynthesis, enzyme regulation, wound-induced responses, and disease responses continues to be generated with tobacco. Readers are referred to a recent review by Aldwin and Lewis (2002) for a detailed summary of such research in tobacco and other species. We will limit our discussion to several examples of events reducing lignin concentration in tobacco, the associated changes in plant morphology, and implications.

An underlying assumption is that to remain agriculturally fit, plants with reduced lignin would need to remain morphologically similar to commercially desirable unmodified crops. Expected responses to significant biochemical or physiological modification of lignin content are reduced vigor and growth. Indeed, such responses have been noted in attempts to modify lignin in tobacco. For example, Pincon et al. (2001) showed reduced plant size in tobacco transformed with COMT and CCoAOMT (caffeoyl-CoA-*O*-methyltransferase) antisense sequences reducing lignin by 48%. Conversely, Zhong et al. (1998) demonstrated significantly reduced lignin (34–59%) in tobacco plants transformed with COMT and CCoAOMT antisense sequences compared with wild-type tobacco, but with normal morphological phenotype. Pincon et al. (2001) observed differences in growth rate under in vitro conditions as compared with greenhouse conditions, leading again to the possibility of genotype \times environment interactions. Chabannes et al. (2001) reported a synergistic interaction of CAD and CCR (cinnamyl CoA reductase) antisense transgenes leading to severe reduction in lignin content in transformants that had a normal morphological phenotype under controlled conditions, concluding that “expression of a specific transgene may have different impact depending on the genetic background.”

To our knowledge, research on modified lignin content in tobacco has been confined to the laboratory or greenhouse, since such tobacco would have no known commercial value at present, other than its important role as a model plant for the study of lignin biosynthesis. However, field tests of lignin-reduced tobacco would be required to determine agricultural fitness, including interactions of lignin reducing mechanisms with environment and genetic background. Sources of reduced lignin in tobacco and their effects on fitness, as determined in laboratory and greenhouse studies, are summarized in Table 3.

Perennial Forages

Fitness of perennial plants in both evolutionary and agricultural terms must consider multi-year survival and clonal reproduction. An excellent review of evolutionary fitness in clonal plants is provided by Pan and Price

(2002) who argue that clonal growth affects fitness through survivorship, future seed production, and daughter ramet production. Because most perennial forage crop varieties are populations they are subject to genetic shifts in agricultural fitness due to these same factors. We will discuss the impact of artificial selection for reduced lignin or increased in vitro dry matter digestibility (IVDMD) on survival and yield of populations of several perennial forage species.

Smooth Bromegrass

A high-IVDMD, low-lignin population and a low-IVDMD, high-lignin population were selected from a single source population of smooth bromegrass (*Bromus inermis* Leyss.) (Ehlke et al., 1986). Seedlings from these two populations were transplanted to fields in three states (Nebraska, Iowa, and Wisconsin) and percentage of surviving individuals recorded periodically for 49 mo (Casler et al., 2002). Little mortality was noted, and no differences in agricultural fitness were attributable to differences in IVDMD or lignin content.

Orchardgrass

Similar high-IVDMD and low-IVDMD populations were successfully selected from a source population of orchardgrass (*Dactylis glomerata* L.) by Rind and Carlson (1988). Seedlings were transplanted to fields in three states (as above), and an inverse relationship of lignin content and IVDMD was assumed, but not measured (Casler et al., 2002). Survival was shown to be related to cycle and direction of selection for IVDMD with 93% survival within the low IVDMD population and 87% survival within the high IVDMD population after four years (Casler et al., 2002). The authors speculate that natural selection in actual high-IVDMD swards could cause a shift to lower average IVDMD in that population over time. Although this research indicates a possible negative relationship of increased digestibility and survival in orchardgrass, the authors did not determine lignin content, nor did they report the strength of the relationship of IVDMD and survival (Casler et al., 2002).

Switchgrass

A source population of switchgrass (*Panicum virgatum* L.) was also selected for high and low IVDMD resulting in a high-IVDMD population and a low-IVDMD population (Vogel et al., 1981). Two additional cycles of selection were conducted for high-IVDMD and the resulting populations were transplanted in a multistate experiment (Casler et al., 2002). A negative relationship between IVDMD and lignin was established. The low-IVDMD population averaged 47% IVDMD and 7.5% lignin while the high-IVDMD population averaged 53% IVDMD and 6.5% lignin. Plant survival and yield were highly correlated to cycle of selection with the high-IVDMD (low-lignin) population exhibiting poorest survival and yield. All mortality occurred during winter months, and effects were most pro-

nounced in the northern-most environment. Vogel et al. (2002) confirmed the above findings on a space-plant population basis, but noted that differential survival was attributable to survival within families, indicating that it should be possible to increase survival in high-IVDMD populations.

Tall Fescue

Chen et al. (2003) successfully decreased lignin content in transgenic tall fescue (*Festuca arundinacea* Schreb.) by downregulation of CAD. No differences between control and transgenic plants were observed for maturity, height, growth habit, tillering, seed yield, lodging, or pest or pathogen susceptibility. The development of these lines is too recent to have data on survival, but the authors indicate such studies are planned. For perennial forage grasses we conclude that although most studies do not measure the direct effect of reduced lignin on agricultural fitness, the effects of selection for IVDMD in some species is indicative of a relationship between reduced lignin, high IVDMD, and reduced survival with the latter at least in part affected by environment. The differential effects of increased IVDMD among species, and among populations within species is again indicative of strong interaction of lignin-reducing traits and genetic background of individuals or populations.

Alfalfa

Using alfalfa (*Medicago sativa* L.) populations divergently selected for high and low lignin content developed by Hill (1981), Kephart et al. (1989) demonstrated associated changes in morphology and yield. The low lignin population exhibited increased leaf to stem ratio. The high lignin population exhibited increased maturity at a fixed date, and increased stem length. Under field conditions, the high lignin population yielded 28% more than the low lignin population. Survivability for 2 yr significantly decreased in the low lignin population versus the high lignin population (34 and 64%, respectively) in a test conducted at a single location (Ames, IA) (Buxton and Casler, 1993).

Transgenic alfalfa with downregulated COMT and caffeoyl CoA 3-*O*-methyltransferase (CCOMT) exhibits reduced lignin content (Guo et al., 2001), while no reduction in lignin quantity was observed in downregulated CAD transgenic alfalfa but lignin composition was altered (Baucher et al., 1999). Data on fitness of such transgenic alfalfa are not yet available.

On the basis of the survival and yield of two divergently selected alfalfa populations, it appears feasible to conclude that reduced lignin can negatively impact the agricultural fitness of alfalfa. However, readers are reminded that in other species results were often variable and dependent on the genetic background of the populations of lines with reduced lignin. Clearly, more varied studies on lignin modification in alfalfa will need to be done before a definitive conclusion on its effect on agricultural fitness can be drawn. Sources of reduced

Table 4. Effects of reduced lignin on agricultural fitness of perennial forages.

Species and source of variation	Effect on agricultural fitness
Smooth Bromegrass Divergent selection for high- and low-IVDMD	no effect on survival or yield
Orchardgrass Divergent selection for high- and low-IVDMD	slight decrease in survival
Switchgrass Divergent selection for high- and low-IVDMD	decrease in survival and yield
Tall Fescue Transformation (downregulation of CAD)	insufficient time to evaluate fitness
Alfalfa Divergent selection for high- and low-lignin Transformation (downregulation of CAD, COMT, CCOMT)	increased leaf/stem ratio reduced stem length and yield insufficient time to evaluate fitness

lignin in perennial forages and their effects on agricultural fitness are summarized in Table 4.

CONCLUSIONS

A common theme throughout this review has been the presence of strong interactions among lignin reducing genes, the genetic background in which they are placed, and the environment in which the resulting plant lines are grown. The combination of all three factors will ultimately determine the agricultural fitness of reduced lignin cultivars and hybrids. Taken as a whole, it appears that reducing lignin content of crop plants can negatively impact their agricultural fitness. However, when evaluating individual events of reduced lignin, effect on agricultural fitness may be neutral or even positive. An example of such a positive reaction to reduced lignin content is provided in a study by Hu et al. (1999) that showed enhanced leaf, root, and stem growth in transformed poplar (*Populus tremuloides* Michx.) with a 45% reduction in lignin.

It is essential that reduction in lignin content, whether resulting from mutations in lignin biosynthesis genes, from selection for traits associated with reduced lignin, or from transformation, be evaluated in diverse genetic backgrounds and gene combinations, and in diverse environments to discover optimal combinations and a true measure of value to, and fitness in agricultural systems.

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