Soybean Stem Lignin Concentration Relates to Resistance to *Sclerotinia sclerotiorum*

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ABSTRACT

Peltier, A. J., Hatfield, R. D., and Grau, C. R. 2009. Soybean stem lignin concentration relates to resistance to *Sclerotinia sclerotiorum*. Plant Dis. 93:149-154.

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is an economically important disease of soybean (*Glycine max*) in the north-central United States and other temperate regions throughout the world. The occurrence and severity of Sclerotinia stem rot in the field is highly dependent upon prevailing environmental conditions, which can prove problematic when evaluating soybean accessions for resistance. The identification of an environmentally stable plant trait associated with resistance to *S. sclerotiorum* could be used to indirectly screen for resistance and would prove useful in the identification and development of resistant germplasm. Observations of the soybean-*S. sclerotiorum* interaction suggest a role for preformed stem lignin content in disease resistance. Although *S. sclerotiorum* produces numerous enzymes that degrade plant cell wall components, no lignin-degrading enzymes have been reported. Despite a hypothesized direct relationship between preformed lignin content and disease resistance, previous studies on soybean have correlated lignin content to nutritional value and not to disease resistance. We hypothesized that plants with low stem lignin are more susceptible and exhibit greater Sclerotinia stem rot severity than plants with high lignin concentrations. Six soybean accessions that varied in response to *S. sclerotiorum* were selected for study in a series of field experiments. Soybean stems were sampled at reproductive developmental stages that correspond to specific events in both soybean plant development and the Sclerotinia stem rot disease cycle. The lignin concentration of stem component samples was quantified. Soybean accessions expressed statistically different disease phenotypes in both 2004 and 2006. Lignin concentrations differed among accessions, growth stages, and plant parts. Results were contrary to our hypothesis, with positively ranked correlations observed between accession Sclerotinia stem rot severity and lignin concentration for all nodes and internodes assayed. For the R3 growth stage, lignin concentration of the internode between the fourth and fifth trifoliate leaves correlated best with disease severity data from each year ($P = 0.005$). These results indicate that resistance is related to low stem lignin concentration and that soybean stem lignin concentration can be used as a biological marker to select for resistance to *S. sclerotiorum*.

Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is an economically important disease of soybean (*Glycine max* (L.) Merr.) in the north-central United States and other temperate regions throughout the world. Early planting date, narrow row width, and high plant populations are key agronomic practices employed to maximize yield potential (35). However, these cultural practices contribute to disease and yield loss in growing seasons conducive to disease development (35,51). Thus, yield potential of soybean is compromised if growers attempt to control SSR by management practices alone. Host

resistance to *S. sclerotiorum* is required for the continued use of modern management practices in regions at risk to SSR.

Although no complete resistance to *S. sclerotiorum* has been identified in soybean, partial resistance was recently identified in both cultivars and plant introductions (2,8,23,27), and has been incorporated into public breeding lines (8). Regardless of cultivar reaction, the occurrence and severity of SSR in the field is highly dependent upon prevailing environmental conditions, which is problematic when evaluating soybean accessions for resistance (1,28,43). Breeding progress is hindered by variable environmental conditions and plant avoidance mechanisms that confound the expression and detection of physiological resistance in field environments. Development of a reliable method for evaluating soybeans for resistance to *S. sclerotiorum* remains a top research priority. The identification of an environmentally stable plant trait that is associated with resistance to *S. sclerotiorum* could be used to indirectly screen

for resistance at both the phenotypic and genotypic level and would facilitate the rapid development of resistant germplasm.

Lignin is a polymer that results from the oxidative coupling of coumaryl, coniferyl, and sinapyl alcohols. Lignin biosynthesis requires multiple enzymatic reactions in three distinct biochemical pathways, the shikimate, phenylpropanoid, and lignin-specific pathways (5). Lignin is a unique natural polymer, being highly heterogeneous and influenced by the abundance of each precursor at the zone of lignification (45). In legumes, lignin is confined to the vascular xylem and tracheary tissues and provides rigidity and resistance to both compressive forces and microbial degradation (42,49).

Lignin content of forage plants is influenced by physiological maturity, genetics, and environmental conditions (25). With few genotype $\times$ environment interactions reported, lignin content is regarded as the most environmentally stable of the measurable forage cell wall traits in alfalfa (4,5,9). In soybeans, lignin concentration is influenced by cultural practices, genetics, maturity, and plant part. High plant populations increase lignin concentration in leaves, while wide row spacing increases lignin concentration in stems (21,46,47). Hintz et al. (22) observed differences in stem lignin concentration among soybean cultivars, which they attributed to differences in maturity groups. Soybean stem lignin concentration is greater in the lower canopy (41), and in alfalfa the proportion of lignified tissue increases over time with stem size, building up progressively in each internode (49).

High lignin concentration is thought to be associated with disease resistance; thus efforts to breed forage crops for reduced lignin concentration may reduce agronomic performance by weakening stem strength or resistance to pests and pathogens (5,50). Lignin is thought to hinder fungal progress by providing a barrier to mechanical penetration, shielding polysaccharide substrates from fungal enzymes, or altering wall components to form an incompatible substrate for fungal enzymes (42). Additionally, lignin displaces water, and may limit the action of ruminant microbes (26) as well as enzymes of plant pathogens.

Despite a hypothesized relationship between preformed lignin and disease resis-

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Accepted for publication 16 October 2008.

doi:10.1094/PDIS-93-2-0149

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tance, most research on lignin concentration has focused on the nutritional value of forages for ruminant livestock (6). Divergent selection has been used to study the role of lignin content in plant survival and disease resistance in smooth brome grass, sorghum, and alfalfa populations. In smooth brome grass lines, high lignin concentration was related to an increase in host resistance against the biotrophic *Puccinia coronata*, but not the necrotrophic pathogens *Pyrenophora bromi* and *Bipolaris sorokiniana* (7). Sorghum lines developed for low lignin concentration did not differ in susceptibility to *Fusarium* or *Alternaria* spp. compared to high lignin lines (12). In alfalfa, lignin concentration affected survival, with 34% of low lignin and 64% of high lignin plants surviving the second growing season (4). In one population, low lignin lines had greater severity of alfalfa rust, caused by *Uromyces striatus*, a biotroph, and spring blackstem and leaf spot, caused by *Phoma medicaginis*, a necrotroph (50). In another population, little association was found between lignin concentration and resistance to *Clavibacter michiganense* subsp. *insidiosum*, *Colletotrichum trifolii*, *Fusarium oxysporum* f. sp. *medicaginis*, *Verticillium albo-atrum*, and *Phytophthora medicaginis* (9).

Observations of the soybean–*Sclerotinia sclerotiorum* interaction suggest a role for preformed stem lignin content in disease resistance. Susceptible interaction phenotypes are typified by long, water-soaked stem lesions that become stringy and bleached with time. Stems of susceptible soybean genotypes often yield to mechanical pressure and collapse. For resistant interactions, lesions are small, superficial, and reddish in color and are restricted to the node area. Different symptoms are revealed upon splitting colonized stems of susceptible and resistant genotypes. In susceptible accessions, the pith is often destroyed and replaced with sclerotia, whereas this is not the case for resistant accessions.

Lignin has many postulated roles in defense against plant pathogens. Similar to resistance to *S. sclerotiorum* (2,23,27), lignin biosynthetic enzymes are polygenically inherited (5). Lignin content may be of particular importance for resistance to SSR, as *S. sclerotiorum* has a rapid growth rate, and many of its pathogenicity and virulence factors are thought to diffuse ahead of advancing hyphae (34). *S. sclerotiorum* produces numerous enzymes that degrade plant cell wall components (19,30–33). However, no lignin-degrading enzymes have been reported for *S. sclerotiorum* (40).

Our objectives were to (i) assess soybean accessions for stem lignin concentration and SSR severity, and (ii) define the relationship between lignin concentration in soybean stems and SSR severity. We hypothesized that plants with low stem lignin are more susceptible and exhibit greater SSR severity than plants with high

lignin concentrations. Results are reported from a series of field studies in which both SSR and stem lignin concentration were evaluated in a set of six soybean accessions that vary in susceptibility to *S. sclerotiorum*.

MATERIALS AND METHODS

Soybean germplasm. Two susceptible and four partially resistant soybean accessions were common to all experiments. The susceptible accessions were BSR-101 and W01.1305, an F5:8 progeny line derived from a cross between the cultivar Dwight (36) and PI 567.323A. The partially resistant accessions were NK S19-90, W04.1000, W04.1001, and W04.1002. The latter three lines were derived from progeny of single plants selected for partial resistance to *S. sclerotiorum* from the plant introductions 561.285B, 561.367, and 567.157A, respectively. BSR-101 and NK S19-90 are commonly used in SSR resistance evaluations as susceptible and resistant checks, respectively.

Experimental design. Experiments were conducted in an SSR disease nursery at the West Madison Agricultural Research Station in 2004 and 2006. Metribuzin (Sencor, 0.42 kg a.i. ha⁻¹; Bayer CropScience; Monheim am Rhein, Germany) and pendimethalin (Prowl, 1.39 kg a.i. ha⁻¹; BASF; Florham Park, NJ) were incorporated with a soil groomer for weed control prior to planting. Soybean seeds were planted to a density of one plant per 2.54 cm of row on 5 May 2004 and 2006. Soybean accessions were planted in a randomized complete block design with four replications in 2004 and three replications in 2006. Plots were planted to 6.1 m lengths and trimmed to 4.9 m after emergence. In 2006, each plot had four equally spaced (76 cm) rows, while in 2004 each plot consisted of five rows, two border rows, and three experimental rows. Row spacing was 76 cm between border and experimental accessions and 38 cm between each experimental row. In 2004, border rows were planted to the susceptible H2627RR (Golden Harvest Seeds, Pekin, IL).

Disease assessment and analysis. Disease was assessed as described by Grau et al. (14). A representative section of each plot was evaluated at the R7 growth stage, and 10 consecutive plants from each of the three experimental (2004) or 15 from the two middle (2006) rows were rated on a 0 to 3 scale: 0 = no symptoms; 1 = lesions on lateral branches only; 2 = lesions on main stem, no wilt, and normal pod development; and 3 = lesions on main stem resulting in plant death and poor pod fill. A disease severity index (DSI) was calculated for each field plot using the following formula:

$$DSI = 100 \times \sum \frac{x}{yz}$$

in which *x* is the ratings, *y* is the maximum rating, and *z* is the number of plants rated.

Lignin quantification. Stems were sampled at reproductive growth stages that correspond to specific events in the development of the soybean plant and the SSR disease cycle. *S. sclerotiorum* infects soybean stems after colonizing senescent flower petals, with symptoms becoming evident at the beginning pod growth stage and progressing to maturity (13). Soybean growth stages R1, R3, and R5 correspond to beginning bloom, pod, and seed, respectively (34). Five asymptomatic plants were sampled from each plot at growth stages R1, R3, and R5.

A preliminary experiment was conducted during 2003 in an SSR disease nursery at the Arlington Agricultural Research Station to identify which soybean stem node most often exhibited symptoms caused by *S. sclerotiorum* (39). According to this survey, of 370 symptomatic stems, 310 had lesions at either the third or fourth trifoliate leaf node. Sampled stems were divided into sections that encompassed these nodes and the surrounding internode tissue. Trifoliate leaf nodes were designated according to the order in which they emerged, beginning with the V1 node or the node from which the first trifoliate leaf emerged. Leaves and pods were removed and nodes V3 and V4 were separated from the surrounding internodes for a total of five stem sections termed Internodes 2-3, 3-4, and 4-5 and Nodes 3 and 4. Node tissue began where the bottom of the petiole began to swell from the main stem and ended at the junction of the petiole and main stem on the top of the petiole. Stem sections were separated by cutting above and below petioles at a right angle to the main stem. Stems were dried in an oven at 55°C for 12 to 36 h; and appropriate internode and node sections from each plot were pooled and ground to pass through a 1-mm screen (Cyclone Mill, Udy Corporation, Fort Collins, CO).

The lignin concentration of nodes and internodes was quantified using the acetyl bromide procedure (10,11,18,24). Stem tissue (~500 mg) was weighed into a 15-ml polypropylene centrifuge tube and subjected to a series of seven extractions to remove cytoplasmic components and pigment constituents that interfere with spectrophotometric analyses. For each extraction, 10 ml of solvent was added to each sample, stirred to suspend plant material, sonicated for 15 min, centrifuged for 10 min to pellet insoluble materials (2,500 × *g* Eppendorf Centrifuge 5810R), and the supernatant was removed by aspiration under gentle vacuum. Solvents used for extractions were ethanol (80%), chloroform:methanol (2:1), and acetone. In sequential order, ethanol (3×) was used to remove small metabolites such as sugars and organic acids, acetone (1×) to remove flavonols and pigments, chloroform:methanol (1× extraction) to remove fatty acids and other membrane components,

and acetone again (2×) to remove chloroform and any remaining water, to aid in sample drying. Samples were air-dried in a fume hood to evaporate solvent (at least 24 h) followed by oven drying (55°C) to constant weight. Each sample tube containing extracted stem tissue was weighed to determine postextraction tissue mass. For each type of stem tissue, a sample (~25 mg) was weighed into a 10-ml glass culture tube (125 × 16 mm) with a screw-top Teflon lined cap and subjected to the acetyl bromide lignin assay.

Acetyl bromide reagent consisting of 25% (vol/vol) acetyl bromide in glacial acetic acid was added (2.5 ml) to each sample and tubes swirled gently to wet the dry stem material. Sample tubes were placed in a 50°C water bath, gently swirled every 0.5 h, and after 2 h submerged in ice to cool samples for safe handling. Approximately 1 ml from each sample tube was dispensed into a 1.5-ml graduated polypropylene microcentrifuge tube using a Pasteur pipette. Samples were spun in a microcentrifuge for 1 min at 14,000 rpm, to pellet undissolved materials, primarily proteins. A 500-μl sample of the supernatant was transferred into a 20-ml glass vial containing: 7.15 ml of glacial acetic acid, 2 ml of 2 M NaOH, and 0.35 ml of 0.5 M hydroxylamine. Controls for background absorbance were handled in the same manner except that no plant tissue was added.

Samples were capped and sealed with Parafilm (Pechiney Plastic Packaging, Menasha, WI) and stored at -4°C until spectrophotometric analysis. A Pasteur pipette was used to place a small aliquot of each prepared sample into a 1-cm cuvette. Absorption spectra (250 to 350 nm) were measured using a scanning spectrophotometer (Beckman DU 800). The control for each set of samples was used for background absorbance and was automatically subtracted from each plant sample scan. For each sample, a plot of absorbance across wavelengths was examined. This was important, as the UV absorption spectrum of a sample can reveal abnormalities caused by excessive degradation, the presence of water, or partially degraded cell wall carbohydrates (especially xylans); all can cause overestimation of lignin concentration (17). For samples that yielded normal UV spectra, the absorbance at 280 nm was recorded. Samples that yielded abnormal spectra were diluted with 10 ml glacial acetic acid and rescanned.

If dilution did not yield a normal plot, the plant sample was rerun with the acetyl bromide method using a different subsample of extracted stem tissue. The extinction coefficient for alfalfa (17) was used to determine the relative lignin concentration of the original dried stem tissue.

Statistical analysis. PROC GLM in SAS (2003, version 9.1; SAS Institute, Cary, NC) was used to analyze 2004 and

2006 DSI data. All lignin concentration data were analyzed as a split-plot model with year as the whole plot and all other terms as subplots using PROC MIXED in SAS (29). Pearson's correlations were conducted in order to properly account for any differences in autocorrelation among adjacent plant parts. As each part was equally related to every other part ($P < 0.0001$), the compound symmetry type of repeated measures model was applied using the repeated statement in SAS. Data were analyzed to test the main effects of accession, growth stage, plant part, and year and to test the accession × growth stage, plant part × accession, plant part × growth stage, year × accession, year × growth stage, year × plant part, and three-way interactions. All factors were considered fixed, except the rep within year and accession × growth stage × rep within year factors. Years were considered fixed effects, as years were not randomly selected but were selected based on the established criterion of favorable disease development. The slice option in SAS was used to aid in determining which of the main effects were responsible for interaction effects.

To determine statistical differences among variables with significant treatment effects, a macro was used to convert mean separation output from PROC MIXED into least significant difference letter designations (44).

As the acetyl bromide method of lignin quantification is very time-consuming, it was important to examine the relationships between accession disease severity and lignin concentration to determine whether lignin concentration of specific plant parts or growth stages allow better relationships than others. To determine relationships between accession disease severity and lignin concentration, Spearman's rank correlation coefficients (r_s) were computed using PROC CORR in SAS.

RESULTS

Disease severity. Although both growing years were conducive for SSR development, disease severity was higher in 2004 than in 2006. In 2004, disease severity indices ranged from 17 to 29 for resistant and from 47 to 52 for susceptible accessions. In 2006, disease severity indices for resistant accessions ranged from 0 to

12 for resistant and from 26 to 34 for susceptible accessions. Differences were detected among accessions in both 2004 and 2006 (2004: $F = 25.53$, $P < 0.0001$; 2006: $F = 26.70$, $P < 0.0001$) (Table 1). The reaction of each accession to *S. sclerotiorum* was similar in 2004 and 2006, and accessions were differentiated according to expected reaction. Significant differences were detected between NK S19-90 and BSR-101, the resistant and susceptible checks, respectively. Additionally, W04.1001 and W04.1002 expressed lower disease severity and thus were rated as more resistant than NK S19-90.

Lignin concentration. All of the lignin concentration data were analyzed using a single model (Table 2). Lignin concentrations differed among accessions (Tables 2 and 3). The resistant check NK S19-90 had the highest concentration and differed from accessions W04.1001 and W04.1000. W04.1000 had the lowest lignin concentration and differed from all other accessions except W04.1001. Lignin concentrations differed among plant parts (Tables 2 and 3). Stem parts closer to the soil tended to have higher lignin concentrations. The third trifoliate leaf node formed (N3) had a higher lignin concentration than node 4 and did not differ from Internodes 2-3, 3-4, and 4-5. The fourth trifoliate node had the lowest lignin concentration and differed from Node 3 and Internodes 2-3 and 3-4.

Lignin concentrations differed between years, with higher concentrations observed in 2006 than in 2004 (Tables 2 and 4). A significant year × plant part interaction was also detected (Tables 2 and 4). This was largely due to the third trifoliate leaf node, which had the highest lignin concentration among stem sections in 2004 and the fourth highest lignin concentration in 2006. In 2004, the Node 3 stem section had a significantly higher stem lignin concentration than the Internode 3-4 and 4-5 and Node 4 stem sections but did not differ from Internode 2-3. The Node 3 and Internode 2-3 stem sections had more lignin than Node 4. In 2006, Internodes 2-3 and 3-4 had significantly higher lignin concentrations than the Node 4 stem section. A striking, although statistically insignificant, trend was observed among internode stem sections in both years. In internode tissue, lignin concentration decreased with

Table 1. Response of soybean accessions to a natural epidemic caused by *Sclerotinia sclerotiorum* at the West Madison Agricultural Research Station in 2004 and 2006

Soybean accession	SSR classification ^y	Disease severity index	
		2004	2006
BSR-101	S - Ck	47 a ^z	26 b
W01.1305	S	52 a	34 a
NK S19-90	PR - Ck	29 b	12 c
W04.1000	PR	25 b	3 c
W04.1001	PR	29 b	3 c
W04.1002	PR	17 b	0 d

^y Sclerotinia stem rot rating: S = susceptible, PR = partially resistant, Ck = check.

^z Values within columns followed by the same letter are not different at $P < 0.05$.

increasing distance from the soil line, with concentrations highest for the Internode 2-3 stem section and lowest for the Internode 4-5 stem section. The fourth trifoliate leaf node had the lowest lignin concentration in both 2004 and 2006.

Lignin concentration differed among growth stages and increased progressively throughout the growing season, with the lowest lignin concentration at the R1 or beginning flowering stage and the highest at the R5 or beginning seed stage (Tables 2 and 5). A significant year $\times$ growth stage interaction was also detected (Tables 2 and 5). However, identical trends were observed in both 2004 and 2006. In 2004, lignin concentrations differed among growth stages, with the lignin concentration increasing throughout the growing season. This same trend was observed in 2006, but concentrations did not differ between the R1 and R3 growth stages.

The only significant three-way interaction was year $\times$ accession $\times$ growth stage (Table 2). The slice option in SAS was used to dissect which factors in the interaction were most responsible for the interaction. This interaction was sliced by the growth stage $\times$ year, growth stage $\times$ accession, and year $\times$ accession interactions. Of most importance, 11 of the 12 slices performed when sliced by the year $\times$ accession interaction were significant. This indicates that growth stages differ for each of these 11 accession $\times$ year combinations; the only exception being W01.1305 in 2006. Upon close inspection of the least squares means, there was a general trend in that nine of the 12 accession $\times$ year combinations followed the same trend as observed with the growth stage main effect. Specifically, the highest lignin concentration occurred at the R5 growth stage and the lowest concentration at the R1 growth stage. The three-way interaction was detected because the lignin concentrations of NK S19-90 in 2004 and W04.1000 and W04.1001 in 2006 were lowest at the R3 growth stage instead of at the R1 growth stage (data not shown).

Table 2. Summary of Type III tests of fixed effects of lignin concentration data using the MIXED procedure in SAS

Effect	P value
Accession (A)	0.0037
Growth stage (GS)	<0.0001
A $\times$ GS	0.1227
Plant part (PP)	0.0020
PP $\times$ A	0.3428
PP $\times$ GS	0.2245
PP $\times$ A $\times$ GS	0.0702
Year (Y)	0.0543
Y $\times$ A	0.4197
Y $\times$ GS	0.0098
Y $\times$ A $\times$ GS	0.0445
Y $\times$ PP	0.0204
Y $\times$ PP $\times$ A	0.5131
Y $\times$ PP $\times$ GS	0.1291

Relationship between SSR disease severity and stem lignin concentration.

Rank correlations of accession means were conducted to determine the relationship between lignin concentration and disease severity. For each plant part assayed at each growth stage sampled, positive relationships were observed between accession rank by disease severity and by lignin concentration. Higher stem lignin concentrations related to higher SSR disease severity (Table 6). For the R1 growth stage, lignin concentrations related best to disease severity in 2004, with significant relationships observed for Internodes 2-3 and 3-4 and Nodes 3 and 4 (Table 6). However, fewer and different relationships were observed in 2006, with the only significant relationships at the Node 3 and Internode 4-5 stem sections. The Internode 3-4 lignin concentration best related to disease severity only in 2004, whereas only a 14% correlation was observed in 2006. Consistency was observed with the Node 3 stem section with correlation coefficients of 0.77 for both growing years. For the R3 growth stage, relationships between accession disease severity and lignin concentration were identical for both growing years (Table 6). Accession lignin concentration of the Internode 4-5 stem section best related to disease severity. Strong relationships were also observed for lignin concentration of Nodes 3 and 4 and disease severity. Poorer relationships were observed between accession disease severity and the lignin concentration of Internodes 2-3 and 3-4. The fewest significant correlations were observed for the R5 growth stage, with only Node 3 in 2006 and Internode 3-4 in both growing years being significant (Table 6).

DISCUSSION

The goal of this study was to address the relationship between preformed lignin concentration of soybean stem sections and the severity of disease caused by *S. sclerotiorum*. The results of this study did

not support the original hypothesis that stated: Plants with low stem lignin concentration are more susceptible to *S. sclerotiorum* and exhibit greater disease severity than plants with high lignin concentration. We observed the opposite, as plants with low stem lignin were more resistant to *S. sclerotiorum* and exhibited less disease severity than plants with high lignin concentration. Han et al. (16) reported similar relationships between disease severity and lignin concentration for three divergently selected smooth bromegrass populations. Han et al. (16) speculated that their results could have been due either to chitin in the plant tissue that confounded their lignin analyses or to lignification that occurred as a result of disease. We studied lignin concentration in asymptomatic soybean stems. Thus, neither chitin nor pathogen-induced lignification should have affected results. It is difficult to speculate on how lignin concentration may affect disease reactions in the smooth bromegrass populations studied by Han et al. (16), as they did not sample asymptomatic plants. An experiment in which fungal tissue is added to asymptomatic plant tissue could help to confirm or reject their hypotheses.

One point of speculation is that lignin is a product of metabolism, and higher levels of lignin are synthesized at the expense of pathways and products associated with host plant defense mechanisms. Blodgett et al. (3) explained that the accumulation of secondary metabolites is dependent upon carbon allocations and the demands on individual biosynthetic pathways by selected metabolic processes. One biochemical response associated with pathogen defense is the accumulation of phytoalexins, low-molecular-weight antimicrobial compounds that are elicited by an invading pathogen (15). The synthesis of lignin and the soybean phytoalexin glyceollin both utilize the shikimate biosynthetic pathway (5). It is likely that as more precursors are shunted into the lignin portion of the

Table 3. Lignin concentration of stem sections of soybean accessions sampled at the R1, R3, and R5 growth stages at the West Madison Agricultural Research Station in 2004 and 2006

Soybean accession	Lignin (g kg ⁻¹ stem tissue)					Accession mean ^y
	Stem section					
	I2-3 ^t	N3 ^u	I3-4 ^v	N4 ^w	I4-5 ^x	
BSR-101	189.2	147.2	190.1	150.3	166.7	168.7 ab
W01.1305	183.1	155.1	178.0	131.3	167.7	163.0 ab
NK S19-90	183.4	153.3	172.3	149.8	160.1	163.8 a
W04.1000	157.1	124.5	146.5	113.7	134.5	135.3 c
W04.1001	164.2	142.1	154.1	120.2	146.7	145.5 bc
W04.1002	163.4	132.1	149.2	114.7	135.4	139.0 ab
Stem section mean ^z	173.4 A	142.4A	165.0A	130.0 B	151.9 AB	

^t Section of internode stem tissue between the second and third trifoliate leaves formed.

^u Section of stem tissue at the third trifoliate node formed.

^v Section of internode stem tissue between the third and fourth trifoliate leaves formed.

^w Section of stem tissue at the fourth trifoliate node formed.

^x Section of internode stem tissue between the fourth and trifoliate leaves formed.

^y Accession lignin values followed by the same letter are not significantly different at $P \leq 0.05$.

^z Stem section lignin values followed by the same letter are not significantly different at $P \leq 0.05$.

pathway, fewer precursor compounds remain for glyceollin production.

Hintz et al. (22) reported that soybean cultivars differ in lignin concentration, but variability in lignin concentration was observed within and among maturity

groups of soybean. However, maturity group is one host trait that does not explain differential reactions of the six soybean accessions used in this study.

Lignin concentration differed among growth stages. Concentration increased throughout the growing season, with the lowest lignin concentration at the R1 growth stage and the highest at R5. Identical results have been reported for both whole plants and stems (21,22). This same phenomenon has been observed in other legumes including alfalfa and red clover (21,37).

Lignin concentrations were higher in 2006 than in 2004. Differences in row spacing may have been responsible for these results. In 2006, rows were spaced 76 cm apart, while in 2004, rows were 76 cm apart between border and experimental rows and 38 cm apart for the three experimental rows. Hintz et al. (22) experienced similar results, with significantly higher lignin concentrations in stem tissue when soybeans were planted at 76 cm row spacing than at 20 cm row spacing. Soybeans have a tremendous capacity to adapt to their environment. Plants grown in wider rows tend to have more stem tissue and higher stem lignin concentrations than plants in narrow rows (19). Narrow rows allow plants to support each other, while wide rows require plants to have greater stem strength to avoid lodging.

Lignin concentrations differed among plant parts. A trend was observed with the internode tissue. Lignin concentration decreased with increasing distance from the soil line. Internode 2-3 had the highest lignin concentration and Internode 4-5 the lowest. Redfearn et al. (41) reported similar results, with higher lignin concentrations in the lower canopy than in the upper canopy. However, this same trend was not observed with node tissue, as Node 4 stem tissue had a higher lignin concentration than Node 3.

Accession lignin concentrations were positively correlated to disease severity, so as lignin concentration increased, disease severity also increased. Lignin concentrations at the R3 growth stage of the stem

section between the fourth and fifth trifoliate leaves (Internode 4-5) correlated most closely to disease severity observed in 2004 and 2006.

The six accessions included in these experiments, although well characterized and differing in susceptibility to *S. sclerotiorum*, are a small sample of soybean germplasm and may not represent the larger soybean genetic pool. A more intensive assessment of the soybean germplasm for stem lignin concentration and reaction to *S. sclerotiorum* is needed before the concept can be established that lower stem lignin concentration is predictive of partial resistance to *S. sclerotiorum*. However, if we were to make recommendations based solely upon this data set, we would suggest sampling at the R3 or beginning pod growth stage at the fourth trifoliate leaf node to form. The acetyl bromide method for quantifying lignin is time-intensive, and these recommendations would significantly reduce the number of samples needed. These recommendations are based upon the statistically significant correlations observed for each of the stem sections sampled at the R3 growth stage. Additionally, at this growth stage, correlations were identical for data collected in both 2004 and 2006. The R3 growth stage is an important stage for the soybean-*S. sclerotiorum* interaction, because by R3, soybean flowers have bloomed and senesced at both the third and fourth trifoliate leaf nodes formed (38). By this time in the soybean life cycle, the flowering growth stages have provided an ample food source for *S. sclerotiorum* to gain entry into the plant (13). Also, lignin concentrations of the fourth trifoliate leaf node were consistently lower than those of all other plant parts in both 2004 and 2006. The potential for lignin quantification as a selection tool to identify SSR-resistant soybean accessions is exciting. The advent of near-infrared resonance spectroscopy, a method with which stem tissue can be analyzed for lignin concentration in a matter of seconds, makes lignin quantification accessible to breeding programs (5).

ACKNOWLEDGMENTS

We thank Peter Crump and Paul Esker for patient statistics consultations. We also acknowledge our funding sources: the USDA Sclerotinia Initiative, the North Central Soybean Research Program, and the Wisconsin Soybean Marketing Board.

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Table 4. Lignin concentration of stem sections of soybeans sampled at the R1, R3, and R5 growth stages at the West Madison Agricultural Research Station in 2004 and 2006

Stem section	Lignin (g kg ⁻¹ stem tissue)	
	2004 ^x	2006
I 2-3 ^y	160.1 abc	187.4 ab
N 3	196.4 a	157.1 bc
I 3-4	144.3 cd	184.8 ab
N 4	113.9 d	147.1 cd
I 4-5	136.4 cd	167.6 abc
Year mean ^z	150.2 A	168.8 B

^x Year × stem section lignin values followed by the same letter are not significantly different at $P \leq 0.05$.

^y Please see Table 3 for stem section definitions.

^z Year lignin values followed by the same letter are not significantly different at $P \leq 0.05$.

Table 5. Lignin concentration of stem sections of soybeans sampled at the R1, R3, and R5 growth stages at the West Madison Agricultural Research Station in 2004 and 2006

Growth stage	Lignin (g kg ⁻¹ stem tissue)		
	2004	2006	Growth stage mean
R1 ^v	92.6 c ^w	137.7 b	115.2 A ^x
R3 ^y	157.7 b	146.3 b	152.0 B
R5 ^z	200.3 a	222.5 a	211.4 C

^v R1, a reproductive stage in which one flower is open at any node on the main stem (37).

^w Year × growth stage lignin values followed by the same letter are not significantly different at $P \leq 0.05$.

^x Growth stage lignin values followed by the same letter are not significantly different at $P \leq 0.05$.

^y R3, a reproductive growth stage in which a 5-mm-long pod is present at a mature trifoliate leaf node on one of the top-most nodes on the main stem (37).

^z R5, a reproductive growth stage; when there is a seed 3 mm long in a pod at one of the four top-most nodes on the main stem (37).

Table 6. Coefficients of rank correlation (r_s) of Sclerotinia stem rot disease severity and lignin concentration data from soybean accessions grown at the West Madison Agricultural Research Station in 2004 and 2006 ($n = 6$)

Growth stage	Year	Stem section analyzed for lignin concentration				
		I 2-3 ^v	N 3	I 3-4	N 4	I 4-5
R3	2004	0.77 ^w	0.89 ^x	0.83 ^y	0.89 ^x	0.94 ^z
	2006	0.77 ^w	0.89 ^x	0.83 ^y	0.89 ^x	0.94 ^z
R1	2004	0.77 ^w	0.77 ^w	0.94 ^z	0.89 ^x	0.66
	2006	0.46	0.77 ^w	0.14	0.60	0.89 ^x
R5	2004	0.71	0.09	0.77 ^w	0.14	0.31
	2006	0.60	0.77 ^w	0.89 ^x	0.43	0.26

^v Please see Tables 3 and 5 for soybean growth stage and stem section definitions.

^w Denotes significance at the 0.0724 probability level.

^x Denotes significance at the 0.0188 probability level.

^y Denotes significance at the 0.0416 probability level.

^z Denotes significance at the 0.0048 probability level.

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