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Comparing Corn Types for Differences in Cell Wall Characteristics and *p*-Coumaroylation of Lignin

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This study was undertaken to compare cell wall characteristics including levels of *p*-coumarate (*p*CA) and lignin in corn (*Zea mays* L.) types. Five different types of corn, four commercial and Teosinte, were grown in the greenhouse in individual pots. For each corn type replicate stems were harvested at tassel emergence. Tissues for cell wall analysis were harvested from stems (separated into rind and pith tissues) and roots. Stem cell wall characteristics across the different corn types were similar for total neutral sugars, total uronosyls, lignin, and phenolic acids. However, the neutral sugar composition of root cell walls was markedly different, with high levels of galactose and arabinose. Levels of *p*CA in the different tissues ranged from 13.8 to 33.1 mg g^{-1} of CW depending upon the type of tissue. There was no evidence that *p*CA was incorporated into cell walls attached to arabinoxylans. Lignin levels were similar within a given tissue, with pith ranging from 86.1 to 132.0 mg g^{-1} of CW, rind from 178.4 to 236.6 mg g^{-1} of CW, and roots from 216.5 to 242.6 mg g^{-1} of CW. The higher values for lignins in root tissue may be due to suberin remaining in the acid-insoluble residue, forming Klason lignins. With the exception of root tissues, higher *p*CA levels accompanied higher lignin levels. This may indicate a potential role of *p*CA aiding lignin formation in corn cell walls during the lignification process.

KEYWORDS: Corn; *Zea mays*; hydroxycinnamates; *p*-coumarate; lignin; suberin; cell wall

INTRODUCTION

Lignin is a macromolecule formed through radical coupling of monolignols synthesized within the cell and transported to the cell wall matrix. Assembly of the lignin molecule is directed by the chemical and physical properties of the individual radicals and the supply of monolignols to specific sites within the wall. Oxidative coupling occurs as the result of wall-bound peroxidases and hydrogen peroxide or in some plants by laccase activity. Grass lignins are uniquely acylated with *p*-coumarates (*p*CA). Although *p*CA can be attached to coniferyl or sinapyl alcohol lignin residues, there is a strong preference for sinapyl alcohol in corn (90%) (*1*). Through structural analysis of corn (*Zea mays* L.) lignin Ralph et al. (*2*) demonstrated that *p*CA is exclusively attached to C-9 (**Figure 1**) of monolignols and not at the α-position (C-7) of monolignols. Attachment at C-7 simply requires that the acid be present during the lignification process and would spontaneously add to a quinone methide formed during radical coupling reactions with no preference for coniferyl or sinapyl alcohols. Because there were no detectable levels of *p*CA attached at C-7, this eliminates the possibility of free *p*CA in the cell wall during lignification. The preference for sinapyl alcohol as the attachment site of *p*CA supports the idea of a metabolically controlled process. Therefore, *p*CA must be transferred to the wall matrix as a preformed conjugate of *p*-coumaryl-sinapyl alcohol (*p*CA-SA) or *p*-coumaryl-coniferyl alcohol (*p*CA-CA). Attachment of *p*CA to the C-9 carbon of either coniferyl or sinapyl alcohol implies enzymatic activity. Recent work has demonstrated the presence of a *p*-coumaroyl transferase in corn stems specifically for transferring *p*CA from its activated form (*p*CA-CoA) to sinapyl or coniferyl alcohol (*3*, *4*). There is a preference for sinapyl alcohol over coniferyl alcohol as the acceptor molecule.

Field corn (*Z. mays* L.) contains relatively high amounts of *p*-coumaroylation on its lignin with levels of 15−20% (*2*). The amount of *p*CA esterified within cell walls can vary (1−4% of cell wall) depending upon the type of grass (*5*). The physiological role for *p*CA in grass cell walls has not been clearly established, although it has been suggested to function as a radical transfer mechanism to aid in lignin formation (*6*, *7*). This study was undertaken to investigate the levels of *p*CA and its relationship to other cell wall components in different corn types that are commercially available. Teosinte was included to determine if selection practices in the development of modern corn types resulted in a shift in cell wall

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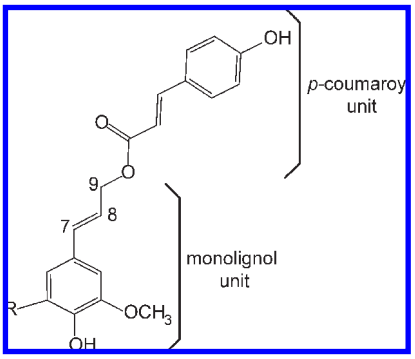

Figure 1. Molecular diagram of *p*-coumaroylation of monolignols in grass lignins. R = H for coniferyl alcohol; R = OCH_3 for sinapyl alcohol.

characteristics, especially *p*CA and lignin. Corn types with different commercial roles were evaluated to compare *p*CA levels to lignin and other cell wall characteristics to determine if there are consistent patterns of *p*-coumaroylation.

MATERIALS AND METHODS

Plant Material. Three replicates of the following types of corn were planted in the greenhouse: B73xMo17 field corn (Brayton Seed Co., Ames, IA), Rainbow corn, Robust White popcorn, Silver Queen sweet corn (all obtained from Carolina Seeds, Boone, NC), and Teosinte (subspeices *mexicana*) (obtained from North Central Regional Plant Introduction Station, Ames, IA). These corn types were selected on the basis of previous work (B73xMo17) or commercial availability of corn types with diverse uses and slightly different growth features (Rainbow corn, popcorn, and sweet corn). Teosinte was included as an ancestral type with a radically different growth and development pattern. The goal was to evaluate this group of corn types to determine their differences in cell wall characteristics. Two seeds of the same type were planted in soil compost/peat/vermiculite (1:1:1) mix in 2 gal pots and culled after 6 weeks to one plant per pot. Each plant was grown under identical greenhouse conditions, given 14 h of supplemental light (high-pressure sodium lamps), fertilized every week (Peter's Professional Soluble fertilizer K:P:N, 20:20:20), and watered twice daily or as needed. All corn plants were harvested when plant development reached tassel emergence. Internodes 8−10 were harvested with rind and pith tissues separated and cut into small pieces (3−5 mm). Internodes were numbered from the soil line to the top of the plant [first six internodes below the soil with seven being the first internode above the soil line (*8*)]. The roots (including primary and secondary fibrous roots) were washed thoroughly to remove all soil and cut into small pieces (4−10 mm). All samples were immediately frozen in liquid nitrogen and freeze-dried before preparation for analytical assays.

Cell Wall Isolation. Dried sample replicates of stems and roots were ground via Udy mill (1 mm), and cell walls were isolated from the total dry matter (*9*). Briefly, plant samples were extracted with cold 50 mM Tris−acetate buffer (pH 6.0) twice prior to starch removal. Samples were suspended in 50 mM Tris−acetate buffer (15 mL 1 g^{-1} of residue) and heated in a water bath (90−95 °C) for 2 h to gelatinize the starch. Amylase (10 IU, Sigma A-3403) and amyloglucosidase (10 IU, Fluka 10115) were added, and incubation was continued for 2 h at 55 °C. Samples were centrifuged at 4000*g* to pellet insoluble materials, and the supernatant was removed. Residues were washed two times with deionized water using centrifugation to pellet insoluble material. After starch removal, residues were extracted with 80% ethanol (two times), acetone (three times), cholorform/methanol (2:1, two times), and once again with acetone. Isolated cell walls were air-dried and stored at room temperature until further analyses. For all plant samples the individual tissues from replicate harvested plants were treated separately except for the pith tissues. Recovered materials after drying and grinding were limited for some plants, so the replicates were pooled before starch removal and cell wall isolation.

Chemical Analysis. Total cell wall hydrolysis was preformed on isolated cell walls using the method of Hatfield et al. (*9*, *10*). Dried cell walls were weighed (100 mg) into 25 × 150 mm culture tubes containing Teflon-lined caps. Cold 12 M sulfuric acid (1.5 mL) was added to each sample along with three to four glass beads (4 mm) and incubated at room temperature for 2.5 h with frequent vortexing. At completion of the 2.5 h strong acid hydrolysis, samples were diluted to 1.6 M using deionized water (diH_2O) and incubated in a heating block (100 °C) for 3 h, vortexing periodically. After incubation, samples were centrifuged (600*g*), and 200 μL aliquots of supernatant were removed and added to 1.8 mL of diH_2O for total uronosyl analysis (*11*). Inositol (10 mg) was added as an internal standard to each tube, tubes were vortexed, and the acid-insoluble residue was collected on a dry, preweighed, glass fiber filter (55 mm, Whatman GF/C) with the aid of a vacuum filtration flask. Collected residues were washed extensively with diH_2O, using a vacuum to assist with filtration. The filtrate (total volume ≈ 200 mL) was transferred to 250 mL Erlenmeyer flasks and saved for neutral sugar analysis. Complete removal of residual acid was ensured by further washing filters and collected residues with diH_2O (total volume ≈ 500 mL of additional water). Excess water was removed from filters via vacuum, and filter disks were placed in a drying oven at 55 °C. After drying for 2 days, the filters were immediately weighed upon removal from the oven, transferred to crucibles, ashed at 500 °C for 5 h, and returned to a drying oven at 55 °C overnight before final weighing. Ten milliliter aliquots of collected acidified filtrate were removed and neutralized with solid barium carbonate. The neutralized filtrate was centrifuged (700*g*) to pellet barium sulfate, and 4 mL of the supernatant was filtered through glass fiber syringe filters (Acrodisc syringe filters, 1 μm, Pall Life Sciences) into 10 mL test tubes and used for neutral sugar assays.

Total Uronosyls. Total uronosyls were determined following the colorimetric procedure of Blumenkrantz and Asboe-Hansen (*11*). Sample size and reagents were scaled down to a 200 μL sample, 1.2 mL of H_2SO_4−borate reagent, and 20 μL of 3-phenylphenol reagent. This decreased the total amount of acid waste generated by this assay.

Neutral Sugar Analysis. Neutral sugars were determined as alditol acetates using the method of Blakeney et al. (*12*). Briefly, 2 mL of the neutralized acid hydrolysate was evaporated to dryness overnight under a stream of filtered air. The dry residue was dissolved in 100 μL of 1 N NH_4OH, and 500 μL of $NaBH_4$ in DMSO (25 mg mL^{-1} DMSO) was added to each tube and capped with a Teflon-lined cap. After incubation at 45 °C for 90 min, the excess $NaBH_4$ was decomposed with 100 μL of glacial acetic acid, and the sugar alcohols were converted to their acetyl derivatives with acetic anhydride (1.0 mL) with 1-methylimidazole (100 μL) as a catalyst. After 20 min of reaction at room temperature, excess acetic anhydride was converted to acetic acid by adding 2.5 mL of diH_2O. Sugar alditol acetates were recovered by extraction with CH_2Cl_2 and quantified using FID-GLC fitted with an SPB-225 column (Supelco, 30 m × 0.25 mm, 25 μm film thickness). Sugars were separated with an isocratic temperature program set at 220 °C.

Cell Wall Hydroxycinnamates. Ester-linked hydroxycinnamates were determined in isolated cell walls using the procedure of Grabber et al. (*13*). The cell wall (CW) residues were accurately weighed (approximately 75 mg) into glass culture tubes (25 mm × 150 mm) and treated with 2 mL of degassed 2 M NaOH. Sample tubes were fitted with Teflon-lined caps and incubated for 20 h at room temperature before acidification with 10 N HCl (pH < 2), and extraction (three times) with 2 mL of anhydrous ether. The combined ether extracts were dried over anhydrous Na_2SO_4, transferred to 2 mL reaction vials,

and evaporated under a stream of filtered air. Total quantities of *p*-coumarates (*p*CA) and ferulates (FA) were determined by FID-GLC using a DB-1 column (30 m × 0.25 mm) (*13*). GLC conditions: injector, 315 °C; detector, 300 °C; temperature program, 220 °C for 1 min, 4 °C min^{-1} to 248 °C, 30 °C min^{-1} to 300 °C, and hold for 20 min.

Nitrobenzene Oxidation. Nitrobenzene oxidation was used to compare monolignol composition among isolated CW samples. The procedure followed the original reaction described by Freudenberg et al. with modifications (*14*−*16*). Briefly, approximately 25 mg of CW was accurately weighed into a stainless reaction vessel, and 6 mL of 2 N NaOH and 0.720 mL of nitrobenzene were added to each reaction vessel. Vessels were tightly closed and heated in a forced-air oven (165 °C) for 2.5 h. After cooling on ice, the contents of each vessel were quantitatively transferred to a 25 mL culture tube with the aid of diH_2O (three times with 5 mL). Isovanillin was added as an internal standard (50 μL of 10 mg mL^{-1} ethanol solution). The basic nitrobenzene reaction mixture was extracted twice with $CHCl_3$ (10 mL) and the $CHCL_3$ extract properly discarded. The aqueous fraction was acidified with 10 N HCl to pH <2.5 and extracted with dichloromethane (CH_2Cl_2, 2 × 8 mL) and anhydrous ether (1 × 8 mL). Extracts were combined, and the volume was reduced under a stream of filtered air to 1−2 mL and stored at −20 °C until analyzed. Samples were placed in 3 mL reaction vials, evaporated to dryness, dissolved in 10 μL of pyridine and 40 μL of silylating agent *N*,*O*-bis(trimethylsilyl) trifluoroacetimide (BSTFA, Pierce), and heated at 60 °C for 30 min. One microliter was injected (split mode 50:1, constant pressure to give an initial flow of 0.77 mL min^{-1}) into a GC-FID (HP 6890 Agilent), and separated on a capillary column (DB-1 50 m × 0.22 mm × 0.25 μm, J&W Scientific) using a temperature program that consisted of an initial temperature of 175 °C (held for 5 min), ramp at 5 °C min^{-1} to 225 °C, and ramp at 10 °C min^{-1} to 275 °C (held for 10 min). Phenolic standards (100 μg of each) *p*-hydroxybenzaldehyde, vanillin, isovanillin, acetovanillone, syringaldehyde, 3,5-dimethoxy-4-hydroxyacetophenone, vanillic acid, syringic acid, *p*-coumaric acid, and ferulic acid, were subjected to the same nitrobenzene procedure to determine response/recovery factors for each.

Determining the Extent of *p*-Coumaryolation of Arabinoxylans in Cell Walls. To determine the amount of *p*CA ester linked to arabinoxylans, the procedure of Marita et al. (*17*) was followed. Cell wall samples (25 mg) were suspended in 0.1 N trifluoroacetic acid (TFA) and heated at 100 °C for 1 h in an 8 mL vial with a Teflon-lined cap. Upon cooling, each acid hydrolysate was filtered through a 25 mm glass fiber syringe filter (1 μm, Acrodisc, Gelman Laboratory). A small plug of silylated glass wool was placed in the bottom of the syringe barrel (3 mL capacity) before the sample was added to prevent the cell wall material from clogging the glass fiber filter. The sample was forced through the filter with the plunger into an 8 mL vial, and the original hydrolysis vial and whole filter apparatus were washed with diH_2O (2 × 1 mL). Free hydroxycinnamates and hydroxycinnamyl-substituted arabinose were concentrated on solid phase extraction columns containing C-18 silica media (500 mg, Envi-18, Supelco) with the aid of a vacuum. Bound material was washed with 1 mL of acidified water (pH <2.5 with TFA) and combined with the flow-through. Bound hydroxycinnamates were eluted from the column with 2 mL of MeOH. Samples were collected in 8 mL vials, capped, and stored at 4 °C until analyzed by GLC-MS (*17*).

Statistical Analysis. All chemical analyses were based on three plant replicates harvested at the same stage of development from a pool of greenhouse-grown corn plants. The exception to this procedure was for pith tissues. For some plants the recovered pith tissue was too small to treat as individual samples and would not allow accurate recoveries after the cell wall isolation procedure. In this case individual harvested plants (replicates) were combined before cell wall isolation. Two replicate assays were run for each chemical analysis. Statistical analyses were performed using one-way and two-way analysis of variance to test the equality of multiple means using variances. The values of the *F* ratios reported were computed from the analysis of variance tables (ANOVA). The significance level being tested was $\alpha = 0.05$.

RESULTS AND DISCUSSION

General Characteristics. Cell walls were isolated from internode tissues of corn stem harvested at tassel emergence. Days to tassel emergence varied among the different corn types, with all plants reaching tassel emergence within 21 days of the earliest developing plant. Teosinte was an exception, not reaching tassel emergence due to our inability to properly control the day length (short days) in the greenhouse environment. Because plants grew best in the greenhouse in mid to late summer months, the light regimen could not be adjusted to accurately control limited daylight conditions. All replicate plants within a given corn type reached anthesis within 1−3 days of each other. The internodes selected for this study were from a region of the plant in which developmental changes had reached a plateau (*18*). Three unique tissues were evaluated from five different types of corn. Teosinte was included to determine if similar cell wall characteristics existed in ancestral type maize. Limited total cell wall characterization work has been done on corn roots, although this is a key structural component for plant function.

Recoveries of cell walls (**Table 1**) from the different tissues of the corn types showed a wide range of recoveries. Cell walls recovered from pith tissues generally ranged from 20.6 to 32.2%. This would be expected as the pith region of corn stems is basically made up of large cells surrounded by a lignified primary cell wall with variable numbers of inner vascular bundles dispersed among them. On a dry weight basis these cells contain large amounts of cytoplasmic contents and relatively small proportions of cell wall material. Recoveries of cell walls from the other tissues were higher, ranging from 58.7 to 81.0%. No attempt was made to separate roots into different types or into separate tissues that generally make up roots because these plants were grown in soil. The small size of the majority of fibrous roots in corn made it impossible to separate roots into separate types of tissues.

A comparison of key cell wall components (**Table 1**) revealed small differences among the corn types within a given tissue type. There were no significant differences in the major cell wall components when Teosinte was compared with the other corn types (**Table 1**). This may indicate evolutionary and genetic selection leading to changes in corn morphology, although the basic cell wall structure has not changed significantly. This comparison is at the gross cell wall component level and does not reflect differences that may exist among the individual molecular components that make up each of the general cell wall fractions (e.g., *p*CA vs FA in total hydroxycinnamates). A comparison among the tissues indicated significant differences in key cell wall components (**Table 1**).

Levels of carbohydrate components within each tissue varied among the corn types, but the patterns were similar. Pith tissues contained the highest levels of neutral sugars and total uronosyls followed by rind and root tissues. Lignin generally followed the opposite pattern, with the least amount of lignin found in the pith followed by the rind and roots. This pattern is expected when components on a

Table 1. Comparison of Major Cell Wall Components Identified in Tissue from Different Corn Types[a]

			mg g^{-1} of CW			
corn type	tissue	CW recovery (mg g^{-1} of DM)	total NS[b]	total UA[c]	lignin	total HCA[d]
Silver Queen	pith	226.2	766.5	54.7	97.9	25.3
	rind	587.0	778.8	33.9	178.4	29.9
	roots	791.7	665.2	34.7	237.6	35.7
Rainbow	pith	276.1	805.4	53.8	116.0	23.0
	rind	712.7	770.3	38.5	203.8	27.3
	roots	772.9	693.8	36.1	230.1	31.5
popcorn	pith	282.5	829.9	57.2	132.0	25.7
	rind	810.3	704.6	32.7	214.8	41.2
	roots	762.9	672.7	30.9	242.6	42.3
B73xMo17	pith	206.1	833.3	59.6	86.1	24.4
	rind	671.1	749.7	26.3	195.1	43.2
	roots	724.4	708.2	37.2	227.0	43.4
Teosinte	pith	322.0	816.3	57.7	96.6	26.3
	rind	800.3	719.9	34.3	236.6	37.7
	roots	790.1	675.5	36.3	234.9	29.8
corn types *F* value		2.8	2.8	0.3	1.8	0.6
tissue types *F* value		159.7*	159.8*	62.4*	103.5*	5.7*

[a] ANOVA two-factor analysis of individual cell wall components compared within tissue types and corn types generally indicated no significant differences among corn type mean levels at $\alpha = 0.05$ within the group but significant differences between mean levels within the tissue types. [b] Total NS = sum of neutral sugars. [c] Total UA = total uronosyls. [d] Total HCA = total hydroxycinnamates (*p*CA + FA + FA dimers). * Significant at $\alpha = 0.05$.

total cell wall basis are compared as these wall components account for 90−95% of the total cell wall. Total hydroxycinnamates accounted for between 2.5 and 4.3% of the total cell wall. In this study no attempt was made to measure the ether-linked ferulates, so total hydroxycinnamates reported here were less than actual levels in the cell wall. On the basis of detailed structural characterization of ryegrass cell walls, there is a proportion of wall-bound ferulates that would not be released by any chemical solubilization method (*19*); therefore, complete quantification is not possible. The proportion of *p*CA in cell walls was similar among types of corn selected for different commercial uses. The hydroxycinnamate, *p*CA, is of interest as it is a major component of grass cell walls, yet the functional role remains unclear. Earlier work demonstrated *p*CA accounted for 15% of the lignin isolated from mature corn stem rind tissues (*2*).

Cell Wall Phenolic Characteristics. The distribution of hydroxycinnamates between *p*CA and ferulates (FA) varied depending on the corn type and tissue analyzed (**Table 2**). Generally, *p*CA represents a greater proportion of the total cell wall hydroxycinnamates in other warm-season grasses (*18*, *20*, *21*). Comparison of corn types and tissues revealed levels of *p*CA ranged from 13.8 to 33.1 mg g^{-1} of CW (**Table 2**). Root tissues and rind tissues were similar in terms of the *p*CA levels within a given corn type. However, the pith tissues consistently had lower levels of *p*CA and lower levels of lignin per unit of cell wall. Although it was not a one-to-one relationship, there appears to be a positive relationship between lignin content and *p*CA levels. This may be expected as the majority of *p*CA in grass cell walls is thought to acylate lignin (*22*). Plotting *p*CA concentration as a function of lignin results in a scatter diagram with no clear relationships (**Figure 1**). However, limiting comparison to pith and rind tissues results in two clusters, a grouping for pith tissues and a grouping for rind tissues. Roots had a less dynamic range for apparent lignin content based on the Klason lignin method, but a wide range of *p*CAs. The dynamic ranges of lignin levels were similar within the pith and rind groups, but the range in *p*CA levels is much broader in the rind tissues versus the pith tissues. Although care was taken to separate the pith and rind tissues, variability may arise in not completely separating one from the other. By comparison of these tissues across corn types, there appears to be a positive relationship between *p*CA content and lignin levels. This suggests more than a passive role of *p*CA integration into corn lignin, reinforcing the idea that *p*CA has an active role in the formation of lignin in grass cell walls (*4*). The higher the lignin contents, the higher the level of *p*-coumaroylation.

The *p*CA levels in root tissues were similar to the stem rind tissue with the exception of Teosinte, which seems to be more in line with the pith tissues (**Table 2**). The Klason lignin method used for analysis will account for all acid-insoluble material including suberin, a structural component of roots. Klason lignin is a standard method accepted for determining lignin and was used in these studies for that reason. Suberin is a hydrophobic composite of phenolic (hydroxycinnamates and monolignols) and aliphatic components coupled through glycerol units (*23*, *24*). Although *p*CA has been found as part of some suberins, it is generally a small fraction of the total mass (*25*). The higher levels of apparent lignin in the root tissues are likely due to suberin and would not resemble lignin found in the above ground portions of the plants. On the basis of developmental studies of corn roots by Zeier et.al (*26*), lignin appears to account for the major portion (approximately 60%) of the materials (lignin + suberin) that would make up Klason lignin in these studies. It is difficult to make a direct comparison to the roots in this study as these were more mature and heterogeneous in developmental stages compared to Zeier et al.'s work (*26*). It would appear that suberin content reaches a maximum in specific root cell types (1.4% of total cell weight), whereas secondary wall

Table 2. Hydroxycinnamates Released from Cell Walls Isolated from Different Types of Corn Germplasm[a]

		mg g^{-1} of CW			
corn type	tissue	*p*CA	FA total[b]	FA	FA dimers
Silver Queen	pith	13.9	10.1	6.9	3.3
	rind	20.7	9.0	5.0	4.1
	roots	22.5	13.2	7.1	6.2
Rainbow	pith	14.5	8.5	5.9	2.7
	rind	20.8	6.5	4.4	2.1
	roots	20.1	11.4	7.2	4.2
popcorn	pith	16.4	9.3	6.3	3.1
	rind	31.9	9.3	5.8	3.5
	roots	27.3	15.0	10.0	5.0
B73xMo17	pith	14.4	11.3	7.8	3.7
	rind	33.1	10.1	6.8	3.4
	roots	33.0	9.1	6.0	3.1
Teosinte	pith	16.4	8.4	6.0	2.5
	rind	29.8	7.9	4.8	3.1
	roots	13.8	18.3	8.4	8.2
corn types *F* value		1.3	0.6	0.5	1.2
tissue types *F* value		5.4*	5.6*	4.9*	5.7*

[a] Cell wall samples were treated with 2 M NaOH at room temperature to release all ester-linked hydroxycinnamates. Analysis by GC-FID as silylated derivatives. ANOVA two-factor analysis of individual cell wall components compared within tissue types and corn types generally indicated no significant differences among corn type mean levels at $\alpha = 0.05$ within the group but significant differences between mean levels within the tissue types. [b] FA total = FA monomers + FA dimers. * Significant at $\alpha = 0.05$.

formation continues in most root cells along with increased lignin accumulation. The Zeier et al. work (*26*) indicated that *p*CA continues to accumulate in developing root cell walls in parallel with lignin accumulation. In addition, they found that there was a shift to higher syringyl content as lignification increased. They concluded that lignification of corn root cell walls mirrored the lignification process in stem tissues.

There does not appear to be a simple accurate method for quantifying suberin, especially in tissues that contain both suberin and lignin such as found in roots. Methods appear to rely on degradation of suberin followed by quantifying the specific aliphatic components (*26*−*28*). Lignin measures in the Zeier et al. work were based on quantifying the monolignols released by thioacidolysis (*29*, *30*). Although such methods give a qualitative picture of lignin, it does not provide for a complete accounting of the lignin as the thioacidolysis releases only ether-linked lignin units (*29*, *30*). Although it is not possible to say how much true lignin was in the corn roots of this study, if one assumed a similar rate of *p*-coumaroylation as seen in stem rind tissues, then the plot of *p*CA versus lignin (**Figure 2**) would fall into a tighter, more consistent relationship. An exception to the general pattern was the Teosinte with levels of *p*CA that were approximately half those of the other corn root samples. It is unclear as to why these roots were so much lower in *p*CA concentrations. It is possible that these tissues have much lower levels of true lignin within their cell walls.

Ester-linked FA generally was at higher levels in the root tissues compared to the rind and pith tissues (**Table 2**). An exception was the field corn (B73xMo17), of which all

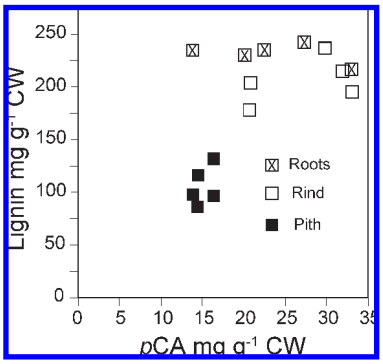

Figure 2. Plot showing the relationship of *p*-coumarate levels to lignin levels in pith, rind, and root tissues from corn.

three tissues had similar levels of FA. More FA dimers were isolated from root tissues across all corn types. Ferulates are a component of suberin, although how it integrates into the overall structure is not clear (*25*). It is possible that attachment to the aliphatic region could lead to feruloyl cross-linking in the form of ferulate dimers (*31*). Ferulates are attached to arabinoxylans, and it is possible that higher levels could be due to increased arabinoxylan incorporation into root cell walls. The amount of arabinose in this tissue was higher, and the ratio of arabinose to xylose was at least twice that of the rind tissues (**Table 3**), indicating potentially more frequently substituted arabinoxylans in roots. It is possible that FA in certain root cell walls acts as a coupling site to anchor suberin into specific wall locations just as ferulates act as nucleation sites for lignification in grass cell walls (*19*). When ferulates become incorporated into lignin, only a fraction of the total cross-coupled ferulates can be released by alkaline hydrolysis methods (*19*, *32*). This leaves a fraction unaccounted for in terms of total ferulates incorporated into plant cell walls. Further structural work must be done to characterize these tissues fully and shed light on the potential roles of higher FA levels in root tissues.

To determine if *p*CA was primarily attached to lignin in the different corn types of this study, cell wall samples from all corn tissue types were treated with a mild acid hydrolysis to release arabinosyl side chains from the arabinoxylans. This treatment effectively hydrolyzes the acid labile α-glycosyl linkage attaching arabinose to xylan, but does not hydrolyze the hydroxycinnamate ester linkage. Analysis by gas chromatography−mass spectrometry of the released components revealed no detectable *p*CA-Ara, only arabinose and FA-Ara (data not shown) indicating, that within the levels of detection, *p*CA is attached to lignin in the pith, rind, and root tissues of corn.

Nitrobenzene Analysis of Tissues. Nitrobenzene oxidation is frequently used as an analytical tool to determine the composition of lignin. Although it does not give a quantitative determination of lignin composition, it does give a relative composition of the monomers found in a given lignin sample. Among the corn types and tissues evaluated, there were differences in lignin composition. The syringyl/guaiacyl (S:G) ratio for root tissues was similar among the corn types with the exception of Teosinte with nearly equal levels of G and S units (**Table 4**). It is interesting to note that Teosinte roots also had lower levels of *p*CA corresponding to lower syringyl content. Rind tissues were more variable for G (0.067−0.106 mmol) and S (0.089−0.162 mmol) units recovered from nitrobenzene oxidation among the corn types. It has been

Table 3. Neutral Sugar Composition of Major Tissues Isolated from Different Corn Types[a]

		mg g^{-1} of CW						
corn type	tissue	Fuc	Rha	Man	Gal	Ara	Xyl	Glc
Silver Queen	pith	0.1	3.0	4.0	4.0	6.7	172.4	766.6
	rind	0.3	0.0	0.8	6.4	18.2	216.3	785.1
	roots	1.0	0.6	1.5	27.3	46.1	218.9	665.2
Rainbow	pith	0.1	2.9	0.2	6.6	6.2	201.9	587.3
	rind	0.2	0.1	nd[b]	4.4	16.6	214.0	535.0
	roots	0.6	0.2	0.3	21.5	38.5	220.9	412.0
popcorn	pith	0.1	1.6	0.1	6.6	7.1	253.7	561.0
	rind	0.1	0.0	nd	3.3	13.6	206.3	481.4
	roots	1.0	0.5	nd	22.7	39.4	220.7	388.4
B73xMo17	pith	0.2	4.5	0.1	9.0	8.5	178.8	632.2
	rind	0.2	0.3	nd	4.6	16.6	229.8	498.3
	roots	0.6	0.2	nd	20.6	40.8	236.5	409.5
Teosinte	pith	0.3	5.7	0.5	8.5	7.6	191.4	602.4
	rind	0.2	0.1	nd	3.5	14.4	218.4	483.2
	roots	1.2	0.5	0.4	28.6	51.5	225.5	368.0
corn type *F* value		1.2	1.1	4.1*	0.8	1.0	0.5	1.3
tissue type *F* value		30.7*	22.2*	2.5	110.3*	156.6*	1.7	84.0*

[a] ANOVA two-factor analysis of individual cell wall neutral sugars compared within tissue types and corn types generally indicated no significant differences among corn type means within the group but significant differences among mean levels within the tissue types. [b] nd, not detected. * Significant at the 0.05 level.

Table 4. Nitrobenzene Oxidation Analysis Results of Rind and Root Tissues from Different Corn Types[a]

corn	tissue	S:G
Silver Queen	rind	0.096
	roots	0.093
Rainbow	rind	0.089
	roots	0.104
popcorn	rind	0.120
	roots	0.106
B73xMo17	rind	0.140
	roots	0.113
Teosinte	rind	0.162
	roots	0.087
corn type *F* value		1.1
tissue type *F* value		0.2

[a] ANOVA two-factor analysis of individual S:G compared within tissue types and corn types generally indicated no significant differences among corn type means or tissue means (significance at $\alpha = 0.05$ level).

shown that the majority of *p*CA is attached to S units within the lignin structure (*1*). Results reported here for *p*CA (**Table 2**) and S:G in rind tissues (**Table 4**) support this observation that higher *p*CA levels coincide with higher syringyl units. Root tissues are much less variable in levels of S units, whereas levels of *p*CA have a wider range (13.8−33.1 mg g^{-1} of CW). It is difficult to draw strong conclusions from the root data as it may not be accurate to assume all of the *p*CA is attached to S units of lignin; nor does nitrobenzene give a complete accounting of all the S units within lignin.

Structural Carbohydrates. As in most herbaceous plants xylose (Xyl) and glucose (Glc) account for a majority of the cell wall neutral sugars in all corn types examined. There were differences in neutral sugar composition among the corn types and tissues. Most of the significant differences were at the tissue level compared to the types of corn. The one exception to this was xylose, which showed no significant differences among corn types or tissue types. It is difficult to know why this would be the case other than arabinoxylans have a key role in cross-linking polysaccharides to lignin through their ferulate side chains. This may represent a key structural feature for cell wall structure and function within the grasses and therefore be more tightly regulated in terms of total amounts incorporated into the wall matrix. Pith tissues were the most unique, with higher levels of uronosyl (**Table 1**) and rhamnosyl residues (**Table 3**) within their cell walls. Because rhamnose is frequently associated with pectic polysaccharides, it is likely that pith tissues are richer in pectin. The majority of cell walls of the pith region contain only thin primary walls. Other cell types like those found in rind and root tissues develop extensive secondary walls. During secondary cell wall development little or no pectin is incorporated into the thickening wall matrix, resulting in a dilution of the original pectic materials associated with the primary wall. Fucose and mannose are also present in many of the tissues and corn types, but levels within the cell walls were low (0.01−0.4%). Roots of all corn types had higher levels of both arabinose and galactose residues in the cell wall material. The arabinose content in particular reached levels 2−3 times the levels found in rind tissues. Root cell walls contained higher levels of ferulates, and it may be possible that xylans of roots are more highly substituted with feruloylated arabinosyl side branches. Because the levels of galactose were also higher, it is equally likely that roots contain significant amounts of arabinogalactans. A more detailed structural analysis of root tissues will need to be conducted in corn to determine the partitioning of individual sugars among structural polysaccharides and their role in cell wall function.

Conclusions. From the results presented here it is evident that morphological changes in overall corn structure are not dependent upon major changes in cell wall characteristics. Across corn types cell wall characteristics were similar within a given tissue, with greater variability among tissues (pith, rind, and roots). Even Teosinte did not deviate significantly from cell wall characteristic patterns of the other corn types. Cell wall neutral sugars and uronosyls levels were similar across corn types within a given tissue. The focus of this work was to investigate unique tissues among different corn types for cell wall characteristics with an emphasis on *p*CA, FA, and lignin. There appears to be a positive relationship between the amount of *p*CA and the amount of lignin within a specific tissue of a given corn type. Because *p*-coumaroylation of lignin is primarily on the syringyl units, it may not be surprising there would be some relationship to lignin accumulation in grass cell walls. If there is a role of *p*CA-SA in aiding lignin formation, then one would expect some relationship between total lignin formation and *p*CA levels within cell walls.

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