Analysis of Plant Cell Walls—Session
Synopsis

JOHN R. OBST, Forest Products Laboratory, USDA-Forest Service, Madison, Wisconsin

Perhaps the most important aspect of plant cell wall research is that of analysis, both in the sense of quantitative determinations of cell wall components and structural characterization of these components. Whether one is investigating the aging of cell walls in corn (Zea mays L.) stover or determining the effect of phenolic acids on the digestibility of grasses, it is readily apparent that appropriate analytical methods are critical to achieve progress.

Although years of research have led to sophisticated and optimized analytical methods, the ultimate analysis of plant cell walls has not been accomplished. No method, or combination of methods, can unambiguously provide complete quantitative analyses of all the chemical components of cell walls. Indeed, it is not likely that such a method will ever be devised.

However, the situation is not necessarily bleak. Many procedures for compositional analysis do provide good estimates of cell wall components and can supply useful data. I stress that these analytical methods provide only estimates. Even if the usual sources of experimental errors are not taken into consideration, virtually all analytical methods have been devised in a functional but simplistic frame. For example, the measurement of polysaccharide content by determination of monosaccharides after acid hydrolysis involves several compromises, but the sugar units that are linked to lignins are never considered. Many believe, as do I, that a number of these covalent lignin-carbohydrate linkages will survive digestion with sulfuric acid. Therefore, these sugar units will not be detected as soluble monosaccharides. However, the amount of sugar units unaccounted for by this reason is trivial for most practical purposes and can be ignored in most sugar determinations. Similarly, the sugar units condensed into the acid-insoluble residue will imperceptibly inflate the corresponding Klason lignin values. Although such lignin-carbohydrate linkages often are not important from the view-

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point of compositional analysis, they can exert a considerable effect on the utilization of plant cell walls.

While thinking about analytical methods, and the interpretation of the resulting data, I was reminded of a story. A traveler, upon returning to his hotel, noticed a very large dog sleeping in the lobby. Although this traveler was fond of dogs, he also was very wary of being bitten. Being careful, he inquired of the hotel clerk, “Does your dog bite?” The clerk replied; “No, he doesn’t.” The traveler then attempted to pet the dog, but as he reached down, the dog viciously bit his hand. The traveler cried out to the clerk, “But you said your dog does not bite!” “But sir,” calmly replied the hotel clerk, “that is not MY dog.” Obviously, it is critical to pose the right question.

A clear understanding of what information is actually needed is critical in choosing an analytical method. The process engineer needs to know the exact yield of glucose after hydrolysis to calculate the cost of production. The scientist who is trying different chemical pretreatments to improve plant digestibility by rumen fluid does not have to be as concerned about the exact sugar contents of the hydrolyzates but does need to be able to accurately determine any changes. Given a choice, a method should be selected on its ability to provide a value as close as possible to the absolute amount of the substance of interest. In many circumstances, however, an absolute analysis is either unobtainable, impractical, or not even necessary. Often, reproducible, relative analyses are sufficient. I must also emphasize that the difficulty in obtaining absolute analytical values should be of foremost concern when evaluating any analytical results.

In choosing an analytical method, consider applying common methods in an unusual manner. For example, alkaline nitrobenzene oxidation has been used to characterize the amount of lignin, as well as lignin monomer composition, from quantity-limited tissue fractions (Obst et al., 1988; Blanchette et al., 1988). Although determining lignin content by nitrobenzene oxidation is not generally recommended, this method was effective in the analysis of laboriously isolated bur oak (Quercus macrocarpa Michaux) tyloses; lignin content of tyloses was about 284 g kg$^{-1}$ (Obst et al., 1988), comparing well with the Klason lignin value of 305 g kg$^{-1}$ for white oak (Q. alba L.) tyloses (Sachs et al., 1970).

The three reviews on analytical methods presented by Theander and Westerlund, Lapierre, and Nevins (see chapters 4, 6, and 5, respectively, in this book) describe a variety of techniques, which range from routine to complex. Although some of these methods are personally recommended and endorsed by the authors, the authors also give some alternative procedures presenting numerous approaches for cell wall analyses. Most analytical methods described, however, are employed for compositional and structural determinations on a macro-scale, unless isolated fractions are obtained from among cell types and cell fragments from various morphological regions. An exception to this is the description of in situ methods for primarily determining the location of polysaccharides in cell walls, as presented by Nevins in chapter 5. These methods include specific staining, oligouronide probes, enzyme-gold cytochemistry, and localization with antibodies.
It may be argued that lignin is the most important structural component of the cell walls of vascular plants. In living plants, lignins provide durability, strengthen supportive tissues, and play key roles in the transport of water, nutrients, and metabolites. Furthermore, the amount, location, and structure of lignin are often considered to be the primary factors that define the utilization of plant cell walls. The excellent work on the distribution of lignin, and its monomer composition, in various morphological regions is discussed by Terashima (see chapter 10 by Terashima et al. in this book). However, the method used, labelling differentiating cells with radioactive tracers and determining the radiolabel by autoradiography, may be compromised by the plant’s reaction to the abnormal growth conditions during the experiment. In addition, the radiotracer method may not be suitable for use in all laboratories, and autoradiographic results should be confirmed by an independent method.

I. LIGNIN DISTRIBUTION IN WOOD

In general, gymnosperms, which include all the common softwood tree species, have normal wood lignins that are predominately composed of guaiacyl (G) units (originating from the precursor coniferyl alcohol). Some gymnosperms, however, contain lignins that are classified as syringyl (S)-G, as a result of the presence of the additional precursor syringyl alcohol. The following section describes lignin distribution in both of these cases: wood cells with G lignins and wood cells with S-G lignins.

A. Wood Cells with Guaiacyl Lignins

Many lignin distribution studies on plant cells have been performed on wood. Lignin can be visualized in light microscopic studies using color reactions such as the Wiesner (phloroglucinol/hydrochloric acid) and Mäule (successive treatments with permanganate, hydrochloric acid, and ammonia) reactions (Sarkanen & Ludwig, 1971). Alternatively, a lignin “skeleton” can be viewed after digesting the specimen with hydrofluoric acid (Sachs et al., 1963; Parham & Cote, 1971). However, few attempts have been made to obtain quantitative results from these methods.

Lignin strongly absorbs ultraviolet (UV) light; UV microscopy was used to determine that the lignin content of Norway spruce [Picea abies (L.) Karsten] middle lamella cell corners was 730 g kg$^{-1}$ (Lange, 1954). This agreed well with the value determined for an isolated middle lamella cell corner fraction from Douglas fir [Pseudotsuga menziesii (Mirbel) Franco] (Bailey, 1936). By similar analyses, the lignin concentration had been estimated at 150 to 160 g kg$^{-1}$ in the cell wall. In general, the thin middle lamella layer has a higher concentration of lignin than does the cell wall. The UV method gives the best results when applied to thin sections (Fergus et al., 1969) and has been used in several lignin distribution studies.
In a unique method for determining lignin distribution, specimens were initially treated with bromine, which reacted with the lignin to provide a tag sensitive to measurement by electron microscopy energy dispersive x-ray analysis (EM-EDXA) (Saka et al., 1978). Although the early results using this technique were not entirely in agreement with the UV findings, a later study (Saka et al., 1982), which accounted for different reactivities of cell wall and middle lamella lignin with bromine, reported lignin distributions similar to those previously determined. The use of a correction factor for differing reactivities resulting from lignin structure has been severely questioned (Westermark, 1985).

Interference microscopy has been used to determine lignin distributions. This method, which can also give information on carbohydrate distribution, was also used to investigate the validity of the bromination correction factor (Donaldson & Ryan, 1987). The conclusions presented further warned that caution must be used when interpreting lignin concentrations obtained by the bromine EM-EDXA method.

To avoid the complication of the nonuniform reaction of bromine with lignin, another EM-EDXA technique was subsequently applied to specimens labelled with mercury instead of bromine. Lignin was uniformly derivatized by treating the specimens with mercuric acetate (Westermark et al., 1988). The results showed that each aromatic ring in the lignin is tagged with one mercuric group, regardless of its morphological location or structure.

The width of the compound middle lamella is about 0.2 µm, which is too small for resolution by most quantitative microscopic methods. Therefore, the much larger cell corner of the middle lamella is usually chosen for quantitative measurements. Similarly, the thick S2 layer of the secondary cell wall is generally used for quantitation. The lignin concentrations obtained by these methods for some woods containing G-type lignin are summarized in Table 7-1. Typically, the middle lamella cell corner was shown to have a considerably higher lignin concentration than does the cell wall. Although some variation occurs in the lignin concentrations in the secondary wall S2 layer (ranging from 200-250 g kg\(^{-1}\) for earlywood), the results from the various methods and laboratories are in relatively good agreement. If these values are correct, and not merely coincidental, and if the species examined are representative, this suggests that the lignin concentration in the S2 layer is rather similar among typical coniferous woods. It would be best, however, to compare the different methods on not only the same species but also the same specimens. Only then would it be appropriate to comment with certainty on the similarity of the lignin concentration in the cell walls of these various species.

Agreement among the values obtained for lignin concentration in the middle lamella cell corner was considerably poorer than that obtained for the cell wall, with values ranging from 480 to 1000 g kg\(^{-1}\) (Table 7-1). Lignin values from tissue fractions isolated from wood and pulps enriched in compound middle lamella content also show considerable variation (Table 7-2). In this case, however, the amount of enrichment for each fraction could not be accurately determined. Although some criticism can be directed at
Table 7-1. Lignin concentration of S2 layer of secondary wall and middle lamella cell corners (CC) of earlywood (EW) and latewood (LW) softwood tracheids.†

<table>
<thead>
<tr>
<th>Specimen</th>
<th>S2</th>
<th></th>
<th>S2</th>
<th></th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EW</td>
<td>LW</td>
<td>EW</td>
<td>LW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway spruce</td>
<td>230</td>
<td>230</td>
<td>550</td>
<td>580</td>
<td>Hg-EM-EDXA</td>
<td>Westermark et al., 1988</td>
</tr>
<tr>
<td>Spruce</td>
<td>200</td>
<td>180</td>
<td>820</td>
<td>820</td>
<td>Interference microscopy</td>
<td>Boutelje, 1972</td>
</tr>
<tr>
<td>Monterey pine</td>
<td>210</td>
<td>--</td>
<td>720</td>
<td>--</td>
<td>Interference microscopy</td>
<td>Donaldson &amp; Ryan, 1987</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>--</td>
<td>920</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loblolly pine</td>
<td>200</td>
<td>180</td>
<td>640</td>
<td>780</td>
<td>Br-EM-EDXA</td>
<td>Saka &amp; Thomas, 1982</td>
</tr>
<tr>
<td>Douglas fir</td>
<td>250</td>
<td>230</td>
<td>830</td>
<td>900</td>
<td>UV microscopy</td>
<td>Wood &amp; Goring, 1971</td>
</tr>
<tr>
<td>Black spruce</td>
<td>230</td>
<td>220</td>
<td>850</td>
<td>100</td>
<td>UV microscopy</td>
<td>Fergus et al., 1969</td>
</tr>
<tr>
<td>Spruce</td>
<td>250</td>
<td>210</td>
<td>460</td>
<td>390-450</td>
<td>UV microscopy</td>
<td>Fukazawa &amp; Imagawa, 1961</td>
</tr>
<tr>
<td>Norway spruce TMP†</td>
<td>250</td>
<td>250</td>
<td>480</td>
<td>480</td>
<td>UV microscopy</td>
<td>Boutelje &amp; Eriksson, 1984</td>
</tr>
</tbody>
</table>

† Variation in lignin concentration, especially for middle lamella cell corners, may have been due to analytical techniques, to natural variation, or both.
‡ TMP is thermomechanical pulp. TMP was used for UV microscopic determination, rather than embedded cross-section. Earlywood and latewood were not separated.
Table 7–2. Lignin concentrations of isolated fractions enriched with middle lamella content.†

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Lignin, g kg⁻¹</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Douglas fir Spruce</td>
<td>714</td>
<td>Micromanipulator</td>
<td>Bailey, 1936</td>
</tr>
<tr>
<td>TMP†</td>
<td>346</td>
<td>Mechanical treatment, sieving</td>
<td>Hardell et al., 1980a</td>
</tr>
<tr>
<td>Disintegrated spruce wood</td>
<td>410</td>
<td>Mechanical treatment, sieving</td>
<td>Hardell et al., 1980a</td>
</tr>
<tr>
<td>Pine kraft pulp</td>
<td>420–560</td>
<td>Mechanical treatment, sieving</td>
<td>Obst, 1982</td>
</tr>
<tr>
<td>Spruce</td>
<td>491</td>
<td>Mechanical treatment, density separation</td>
<td>Sorvari et al., 1986</td>
</tr>
</tbody>
</table>

† Calculated for untreated wood.
‡ TMP is thermomechanical pulp.

each technique, the sources of disagreement are not clear. Particularly interesting are recent studies that indicate the occurrence of nonlignified and partially lignified middle lamella cell corners.

Results of a study on permanganate-treated wood have a potentially great impact on the interpretation of lignin distribution studies. Electron microscopy of birch (Betula papyrifera Marsh.) cross-sections treated with permanganate showed that the cell corners frequently were not electron dense; that is, they were unstained by permanganate and therefore not lignified (Blanchette et al., 1989). In another study, lignin distributions were carefully determined by mercurization EM-EDXA, permanganate staining, and immunocytochemical labelling (Daniel et al., 1991). Although birch (B. pendula Roth = B. verrucosa Ehrh.) was primarily examined, it and a range of other angiosperm and gymnosperm woods were commonly observed to have nonlignified middle lamella cell corners. These nonlignified areas were not intercellular voids, which occur frequently in compression wood and much less frequently in normal wood. Voids and nonlignified cell corners could be distinguished clearly from each other. (Note: compression wood is the reaction wood formed by gymnosperm on the lower side of displaced stems and branches. For a thorough discussion of the unique chemistry and anatomy of compression wood, see Timell, 1986). These reports are truly remarkable in the context of the many microscopic examinations of wood that did not reveal the apparently extremely heterogeneous distribution of lignin in middle lamella cell corners. In this context, it is interesting to note that when the bromination EM-EDXA technique was applied to two specimens of Monterey pine (Pinus radiata D. Don), sites in one of these trees were less brominated (that is, had less lignin) in the cell corners than in the corresponding secondary walls (Donaldson & Ryan, 1987).

In addition to cell corners with nonlignified regions, middle lamella cell corners have been found to have a complete range of levels of lignin content (Daniel et al., 1991), suggesting that the normal signification process in developing cells, which begins in the cell corner, had been interrupted. Furthermore, the variations observed in the chemical composition of cell corner
regions, if unaccounted for, could seriously compromise the results and con-
clusions reached from previous lignin distribution studies. Additional work
is required to assess how widely lignin concentration varies among and with-
in species, and what effect this variation may have had on past results.

Although still in developmental stages, the Raman microprobe, an in-
situ technique that provides information on areas as small as 1.6 µm, has
been used in the study of woody tissues (Agarwal & Atalla, 1986). Earlier
studies indicated that the concentration of lignin varied within the secon-
dary walls of the tissue. Recent work has shown that this observation needs
to be further analyzed in light of the fact that lignin contributions to the
Raman spectrum are affected by both the resonance Raman (RR) and con-
jugation phenomena, and that the former contribution declines in time (Bond,
1991). When band-intensity enhancement caused by the RR effect was re-
moved, the intensity data represented lignin concentration much more ac-
curately. Using this approach, lignin concentration in the latewood middle
lamella cell corner of black spruce was found to be twice that of its value
Although the spatial resolution capability of the Raman microprobe does
not come close to that of the UV microspectrophotometer, its appeal lies
in the fact that the information is obtained through vibrational states in lig-
nin. Vibrational modes, as opposed to electronic transitions, are better suit-
ed for quantitative studies of one component in a multicomponent system.

B. Wood Cells with Syringyl-Guaiacyl Lignins

Gymnosperms with S-G lignins include species in the Gnetophyta and
the Podocarpaceae (in the Coniferophyta) (Sarkanen & Hergert, 1971). In
contrast, angiosperm woods usually have S-G lignins, but their S/G ratio
can vary considerably. For example, some hardwoods, such as madrone (Ar-
butus menziesii Pursh), have a S/G ratio of 2.3 whereas boxelder (Acer negun-
do L.) has a S/G of only 0.2. These values represent extremes, however, and
most temperate zone hardwoods have S/G ratios closer to 1. Tropical hard-
wood species are much more numerous than hardwood species of the tem-
perate zone; a wide range of S/G values has also been found for the lignins
of tropical hardwoods. Relatively few of these species, however, have been
characterized and “typical” S/G ratios can neither be suggested nor, perhaps,
should they.

Whether the lignin is composed of G units or a mixture of S and G units
is germane to the determination of lignin distributions. Neither the UV
microscopic method nor the bromination EM-EDXA method can be direct-
ly applied to specimens that contain lignins with significant S contents. Syrin-
gyl units absorb approximately three times less UV light than to G units,
and S units react with bromine to a greater extent (1.2 times) than do G units
(Saka et al., 1988). If S-G lignin units were intimately mixed either as copolymers or homopolymers, these lignin distribution methods could be ap-
plied simply with appropriate correction factors. The distribution of S units,
however, may not be homogeneous within the lignin. If such is the case, the
S/G ratios must be known before the lignin distribution can be determined by either of these methods.

The S-G compositions of birch wood lignin reported in several morphological regions are given in Table 7-3. These values were used to determine lignin distribution in the studies cited in the table. Because S and G units react to the same extent with mercuric acetate, the mercurization EM–EDXA method would appear to be best for determining the lignin distribution of tissues containing S-G lignins (Eriksson et al., 1988). The relative lignin concentrations in birch woods determined by three methods are summarized in Table 7-4. Agreement among the methods is not exact. Note that the cell corner lignin content determined by UV microscopy is 1.4 times greater than that determined by the bromination method. It is also important to note that in recent reports, the cell corner regions displayed a wide variation in lignin content (Blanchette et al., 1989; Daniel et al., 1991).

Angiosperm wood fibers containing S-rich lignin in their cell walls but G-rich lignin in their middle lamella cell corners and vessel cell walls were described further for several species (Musha & Goring, 1975). The authors concluded that as the methoxyl/C-9 value for the total lignin increases, the S content of the fiber and ray parenchyma secondary wall lignin also increases. Although UV microscopy showed that vessel cell walls contained mainly G lignin, the S content of the vessel cell wall lignin was found to increase at high methoxyl/C-9 values. This method, however, required a major assump-

<table>
<thead>
<tr>
<th>Morphological region</th>
<th>Syringyl and guaiacyl content or ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV microscopy</td>
</tr>
<tr>
<td>Fiber S2</td>
<td>Syringyl</td>
</tr>
<tr>
<td>Vessel S2</td>
<td>Guaiacyl</td>
</tr>
<tr>
<td>Ray secondary wall</td>
<td>Syringyl</td>
</tr>
<tr>
<td>Middle lamella cell corner†</td>
<td>50:50</td>
</tr>
</tbody>
</table>

† Ratio of syringyl to guaiacyl.
‡ Between fibers.

Table 7-3. Syringyl-guaiacyl composition of birch lignin in various morphological regions.

Table 7-4. Lignin distributions in white birch determined by mercurization EM-EDXA (Eriksson et al., 1988), UV microscopy (Fergus & Goring, 1970), and bromination EM-EDXA (Saka & Goring, 1988).

<table>
<thead>
<tr>
<th>Morphological region</th>
<th>Relative lignin concentration†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hg-EM-EDXA</td>
</tr>
<tr>
<td>Fiber S2</td>
<td>1</td>
</tr>
<tr>
<td>Vessel S2</td>
<td>1.5</td>
</tr>
<tr>
<td>Ray secondary wall</td>
<td>1.6</td>
</tr>
<tr>
<td>Middle lamella cell corner†</td>
<td>3.0</td>
</tr>
</tbody>
</table>

† Values given in parentheses give lignin concentration (g kg⁻¹).
‡ Between fibers.
tion, namely that the relative distribution of lignin was the same for all softwoods and hardwoods ("the concentration of lignin in a particular morphological region divided by the lignin content of the wood is constant;" Musha & Goring, 1975). This assumption is in conflict with the previous observations that the concentration of lignin in the middle lamella region of hardwoods is greater than that in softwoods and that the lignin concentration in the cell walls of hardwoods is less than that found in softwoods (Sachs et al., 1963).

Characterization of lignins isolated from fiber and middle lamella enriched fractions obtained from kraft pulps also provided conflicting evidence. Although the data suggest that birch and sweetgum (*Liquidambar styraciflua* L.) middle lamellae contained G- and S-rich lignins in their cell walls, a more uniform S/G ratio was found for the lignins in American elm (*Ulmus americana* L.) and red oak (*Quercus rubra* L.) (Obst & Landucci, 1986). These results are consistent with an earlier histochemical study of lignin distribution in bark and wood cells of 74 angiosperm species (Srivastava, 1966). Based on Wiesner and Mäule tests, a high degree of variability was observed and the conclusion was drawn that lignin monomer composition may or may not vary among species and among different cells of the same plant.

Recently, additional studies of lignin monomer-type distribution in angiosperm woods using UV and visible microspectrometry resulted in novel observations. Ultraviolet microspectrometry of Japanese beech (*Fagus crenata* Blume) earlywood vessels and fibers gave results similar to those found earlier (Fergus & Goring, 1970). Latewood tissue, however, was found to be quite different. Vessel walls contained a considerable proportion of S moieties whereas the S/G ratio of the fiber cell wall lignin was lower than that of the earlywood (Takabe et al., 1992). The authors also made the startling observation that the S/G distribution in Japanese beech varied within one annual ring. The red Mäule reaction, which is positive only with S units, confirmed the UV microspectrometry results. Earlywood vessel walls gave only a weak absorption at 515 nm, but latewood vessel walls gave an appreciable absorption. All fibers in the annual ring gave a strong absorption, but the earlywood fibers were more intensely stained than the latewood fibers, although the terminal zone gave the most intense color.

Ultraviolet and visible microspectrometry were used to characterize the monomer composition of 25 species of woody angiosperms (Wu, J., K. Fukazawa, and J. Ohtani, 1992. Distribution of S and G lignins in hardwoods in relation to habitat and porosity form in wood. Submitted to Holzforschung). This investigation showed that diffuse-porous woods had vessel cell walls that contained G-rich lignin, whereas ring-porous woods had latewood vessel walls that were Mäule positive (S-rich) and earlywood vessels that were Mäule negative. Woods that tested positive for S units in latewood vessels included *Quercus acutissima* Carruth., *Q. yunnanensis* Franch., *Catalpa duclouxii* (Dode) Gilmour, and *Fraxinus lanunginosa* Koidz. var. serrata Hara. The vessels of *Q. aquifolioides* Rehd. et Wils. in Sarg., which are arranged in a radial pattern, gave a weak Mäule reaction in earlywood and latewood.
Some lignin and S/G distribution studies that employ microscopic methods end with a caveat that goes something like this: these results need to be confirmed by analysis of isolated fibers, vessels, and middle lamella fractions. Any such pure fractions would be ideal candidates for analysis, including those techniques described by Theander and Westerlund and Lapierre (see chapters 4 and 6, respectively, in this book). Several schemes have been developed in this pursuit. Because unaltered pure fractions can rarely be obtained, the results from such studies have not been conclusive.

For example, one study claimed to provide a pure middle lamella fraction with a high \( p \)-hydroxyphenyl lignin from black spruce \([\text{Picea mariana (Miller) B. S. P.}]\) (Whiting et al., 1981). The results of a later study suggested that this lignin was actually compression wood lignin (Westermark, 1985). Furthermore, the middle lamella of normal softwoods is composed primarily of G units. Many other techniques have been used to prepare enriched middle lamella and cell corner fractions (for example, Harden et al., 1980a, b; Obst, 1982, 1985; Sorvari et al., 1986), but it is not clear whether the desired fraction is always obtained in high purity.

An isolation that provided unadulterated vessels from white oak (Obst, 1982) and red oak (Obst et al., 1987) clearly showed the S nature of the lignin of these cell walls. Yet, even this study may have been compromised by the chemical pretreatment required to liberate the vessels from the wood. It is interesting to note that these isolated vessels were the large earlywood cells that might have been expected to contain G lignin, based on the recent microspectrometry results (Takabe et al., 1992; Wu, J., K. Fukazawa, and J. Ohtani, 1992. Distribution of S and G lignins in hardwoods in relation to habitat and porosity form in wood. Submitted to Holzforschung). These results suggest that it would be worthwhile to apply the microspectrometry methods to the North American oak species to investigate further the variation of the S content across an annual ring.

Some basidiomycete fungi degrade hardwoods in an usual pattern to produce rotted wood with a white, stringy appearance. These decay-resistant “strings” are vessel elements. The fibers and rays are conveniently removed by fungal digestion, leaving behind a relatively pure vessel fraction. Although partially degraded by the white rot fungus \( \text{Phellinus kawakamii} \) Larson, Lombard, et Hodges, vessel strings were isolated from the wood of \( \text{Acacia koa} \) Gray var. \( \text{koa} \) and subjected to analysis by alkaline nitrobenzene oxidation (Blanchette et al., 1988). The results clearly showed that these vessels were G-rich compared to the sound whole wood, but it was also clear that their lignin contained a significant amount of S units. Because fungal degradation preferentially removes S units in the earlier stages of decay, it is possible that the S content of the intact vessels was actually greater. This same study reported a significant S lignin component in fungally enriched vessel fractions from \( \text{Tilia} \) (basswood) and \( \text{Acer} \) (maple) spp.

Perhaps one of the most pure fractions ever isolated from an angiosperm wood was that of the separated tyloses from bur oak (Sachs et al., 1970). These tyloses were mechanically removed from the wood and then analyzed by alkaline nitrobenzene oxidation (Obst et al., 1988). The tyloses, which
are balloon-like outgrowths of parenchyma cells into the lumens of adjacent vessels, contained a S/G lignin, but it was G-rich compared to both the total lignin and isolated parenchyma (ray) cells. Unfortunately, no microspectrometric study has yet been done on these tyloses in situ.

Even when whole cells are isolated, such as the vessels described in this report, or fibers and tracheids that have been carefully screened and sorted, lignin characterization is performed on samples containing both cell wall and middle lamella lignins. Further concentration of cell wall lignin, however, has been attempted by chemically removing the middle lamella from such fractions by oxidative delignification (Obst, 1982).

II. LIGNIN DISTRIBUTION IN NONWOOD PLANTS

The perennial grass bamboo (Bambuseae) is a significant raw material in developing nations, where it is used for the production of pulp and paper. It is surprising, therefore, that “no comprehensive research has been conducted so far on the nature of lignins in bamboos and technical bamboo lignins” (Tai et al., 1990). Bamboo has been shown to contain a S-G lignin that also contains a large amount of ester-linked \( p \)-coumaric acid (Higuchi et al., 1967; Nakatsubo et al., 1972). The lignin of \textit{Phyllostachys pubescens} Mazel ex H. De Lehaie was characterized and found to contain \( p \)-hydroxy-cinnamic acid, G, and S units in the molar ratio of 1:4:3 (Tai et al., 1990).

When \textit{Phyllostachys makinoi} Hay was extracted with NaOH, the cell wall substance was reportedly removed from the secondary walls, whereas extraction with trifluoroacetic acid primarily removed substance from the compound middle lamella (Fengel & Shao, 1984). The lignin in these extracts was subsequently characterized, and the secondary wall lignin was shown to have considerably lower syringyl content than that of the compound middle lamella (Fengel & Shao, 1985). Although this result is exactly the opposite of what would be predicted from the S/G distribution in birch, the isolated bamboo lignins may have undergone significant degradation, which may seriously compromise interpretation of the data.

The cell wall structure of 26 nonwood plant fibers, including bast fibers, leaf fibers, seed hairs, and fibers of grasses (Gramineae), were examined by transmission electron microscopy to investigate layer composition, microfibril orientation, and lignin distribution in the secondary wall (Nanko et al., 1983). Lignin distribution and concentration in alfalfa (\textit{Medicago sativa} L.) stems was investigated by staining under the Wiesner reaction conditions, followed by image analysis (Kuehbauch, 1985). The image analysis results were correlated to the chemically determined lignin content of the total stem and to in vitro digestibility. The authors suggested that quantitative image analysis could be applied to plant tissues to provide both lignin distributions and contents while only requiring a small amount of tissue for analysis.

The challenge to account for differing lignin monomer compositions in various morphological regions also occurs when determining lignin distributions of forage plants. For example, sclerenchyma walls from the lower in-
ternodes of bermudagrass [*Cynodon dactylon* (L.) Pers.] stained positive with the Wiesner reaction but did not react with chlorine-sulfite, indicating the presence of G-type lignin; parenchyma walls did not react under the Wiesner conditions but gave a positive chlorine-sulfite reaction, indicating the possibility of a S-type lignin (Akin, 1989). However, the significance of the histological staining remains to be verified.

Although the phenolic and lignin contents and distributions in forage plants have not yet been exactly correlated to digestibility, the influence of these components on limiting digestibility by ruminants is unquestioned. Qualitative distributions of phenolics have been performed by treating bermudagrass with diazonium salts. The different colors produced by specific compounds allowed identification of phenolic types within cell walls (Akin et al., 1990).

The walls of bermudagrass sclerenchyma and parenchyma cells were further examined by UV absorption microspectrophotometry (Hartley et al., 1990). By this technique, the concentration of ferulic acid in sclerenchyma walls from the fifth internode from the apex was determined to be 109 g kg\(^{-1}\). Although the relationship between a positive Wiesner reaction and UV absorption was not established, cell walls that had high UV absorbance also stained for lignin with the Wiesner reaction. Additionally, these walls were resistant to digestion by rumen microorganisms. Cell walls that had low UV absorbance and gave negative Wiesner reactions were either completely or partly digested. Microspectrometry of forage plants will undoubtedly be expanded and refined because this method has potential to provide a rapid assessment of the nutritional characteristics of forage cell walls.

### III. CONCLUSION

The analysis of the chemical constituents of plant cell walls and the structural characterization of these components are critical undertakings in efforts to improve plant cell wall utilization. Therefore, it is necessary to develop and use appropriate analytical methods. Because ultimate analytical methods have not been devised, investigators must interpret with caution not only their own data but also that of others.

The distribution and composition of lignin, primarily in wood tissue, was reviewed in this chapter. Several experimental techniques presented a range of lignin concentration values. None of the experimental techniques is beyond criticism, including some key assumptions made in various studies. More recent results suggest that lignin concentrations in the middle lamella cell corners may be extremely variable, and this could be a factor leading to the observed disagreement in findings. Of all the methods used for determining lignin distributions, the mercurization EM-EDXA method appears to be the most suitable. This method has not been used widely, however, and it remains to be thoroughly investigated.

Perhaps just as important as determining lignin distributions is elucidating the variation of the lignin monomers in different morphological regions.
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Such information could be useful in explaining many chemical and physical properties of lignified plant cells. The results to date indicate that it is doubtful whether a “typical” S/G distribution can be defined. Rather, a range of distributions may be likely, even though some generalizations may be valid. Considering only angiosperm woods, this is not surprising. Because angiosperm trees vary greatly in form, leaves, anatomy, and chemistry, would it not be expected that their lignin concentrations and lignin monomer distributions would also vary?

The distribution of lignin and lignin monomers in nonwood plants is also likely to be complex and variable. Because of their functionalities and reactivities, however, these components might be specifically derivatized and mapped, providing even more information about the composition of cell walls.

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