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Within tree variability of lignin composition in *Populus*

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Abstract Clonal variability among trees has been studied and found to have profound effects on nearly all measured phenotypes. However, when estimating wood properties it is important to consider variability within the tree. The position in which a tree is sampled could have a large influence on biomass characterization. We looked at variability in lignin content as height increases and as the number of rings from the pith increase in *Populus* species. Seven trees were destructively sampled; subsamples were obtained along a 2.4 m length of each stem and across increment rings. All samples were analyzed by pyrolysis molecular beam mass spectroscopy to map the variability across sampling heights and/or ring positions in lignin content. The results of this study indicate that when sampling a tree, there is more variability from ring to ring than at different heights going up the stem.

Introduction

Wood quality has long been an important focus area for tree breeders and tree improvement programs. For years tree breeders and geneticists have worked to

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improve growth characteristics such as height, volume, specific gravity, and tree form (Dinus et al. 2000; Kopp et al. 2001; Riemenschneider et al. 2001). Significant gains have been made on improving these characteristics on many different species through tree breeding (Apiolaza and Garrick 2001; Borralho et al. 1993; Fries 1987; Lambeth et al. 1994; Li et al. 1999).

Conventional assessment of wood properties in a tree usually requires wood disks or wood cores to be obtained, though wood disks are rarely used due to the destructive nature of sampling. Thus, wood cores have become a general standard for assessing wood properties of a tree whether by means of traditional wet chemistry methods or a more rapid technology such as Silviscan or near-infrared spectroscopy (Downes 1997; Schimleck et al. 2004; So et al. 2004; Tuskan et al. 1999). Wood cores are preferred due to their non-destructive nature and the relatively short period of time required to obtain samples. Wood cores allow breeders to assess progeny at young ages with little damage, while leaving the trees to grow for assessment at rotation age. Wood cores, however, can suffer from sampling bias.

Wood cores are typically taken at diameter at breast height (DBH) or approximately 1.3 m and represent a very small point sample relative to the total volume of a tree. Several studies have successfully related DBH wood core samples to whole-tree data for several physical wood properties (basic density, fiber properties, etc.) (Igartua et al. 2003; Ona et al. 2001; Raymond and Muneri 2001; Raymond et al. 2001). The relationship of wood cores to whole-tree values has been studied for physical wood properties, but there is a general lack of information on this relationship for chemical wood properties.

Estimating the natural variation of wood properties within a tree is critical in determining the appropriate selection intensity within a population as well as in the design of appropriate and accurate sampling schemes. For traditional breeding, large amount of natural variation among trees form the basis of artificial selection that attempts to shift the mean of the breeding population. However, if there is a large amount of within tree variation, sampling height and age may affect which individuals are selected for the next breeding cycle. Characterizing the relationship between wood core properties and whole-tree properties would enable traditional breeding. Moreover, researchers working with transgenic trees may want to create individuals that are outside the natural variation of the population. Unless the range of natural variation within a species is known, it is difficult to accurately assess the population for selections.

The height from which a core is extracted and/or the specific ring that is sampled within a tree may have large effects on the outcome of wood property analysis (Gartner et al. 2002; Raymond and Muneri 2001; Sykes et al. 2003). Ring effects have a measurable impact in determining at what age to assess wood properties and thus affect the amount of time required for breeding cycles (Lambeth 1980). The earlier a tree can be selected for breeding, the faster the next generation of trees can be produced and more gain can be realized per unit time. These age-age correlations can vary greatly depending on which wood property is of interest and have a tremendous impact on when breeding programs assess their progeny tests (Gwaze et al. 2002; Osorio et al. 2003; Xiang et al. 2003).

This study will examine the variation in lignin content determined from pyrolysis molecular beam mass spectroscopy (pyMBMS) associated with different heights up the tree and variation from the pith to the bark of *Populus*. Diameters were also measured to determine if the diameter had a significant effect on the variability of wood properties.

Materials and methods

Plant materials and wood samples

Wood disks were sampled from seven different poplar (*Populus* spp.) clones at five different heights of 0.3, 0.6, 1.2, 1.8, and 2.4 m from the base of the stem. Although the trees were taller than 2.4 m, the sampling was terminated at this height due to the smaller trees having less than five rings from pith to bark. Also, due to sampling from previous experiments, all five wood disks were not available for all the trees in this study. The trees were seven years old when harvested and had varying diameters (6.4–24.1 cm). All seven trees were grown at Clatskanie, OR under conventional short-rotation silviculture (Tuskan 1998).

Characterizing variation in lignin content of each tree at different heights involved removing a single ring from each of the wood disks at each height in order to minimize variation. The tree with the smallest diameter had five rings from pith to bark and, therefore, Ring 5 was subsequently selected for all height-to-height comparisons. This ring was ground with a FOSS Cyclotec grinder and duplicate pyMBMS spectra were obtained for estimation of the wood properties for all the wood disks.

A simulated "increment core" 12 mm wide from the pith to the bark was removed from the north side of each wood disk taken at a height of 1.2 m to represent DBH. This wood core was then processed as described above. Duplicate pyMBMS spectra were obtained for each ring and were used to estimate lignin content and syringyl to guaiacol ratio (S:G). In order to verify the results obtained from analyzing the rings from pith to bark, the individual ring analysis was repeated on the wood disks taken at 0.3 and 1.8 m heights on the stem.

Pyrolysis molecular beam mass spectrometry

The ground wood samples were weighed in quartz boats to approximately 20 mg and pyrolyzed in a reactor consisting of a quartz tube (2.5 cm inside diameter) with helium flowing through at 5 L/min (at STP). The reactor tube was placed such that the sampling orifice of the molecular-beam mass spectrometer was inside the end of the quartz reactor (Fig. 1). A custom-built molecular-beam mass spectrometer with an Extrel Model TQMS C50 mass spectrometer was used for pyrolysis vapor analysis (Evans and Milne 1987; Tuskan et al. 1999). The reactor was electrically heated and its temperature maintained at 500°C. Total pyrolysis time was 90 s, although the pyrolysis reaction was completed in less than 50 s. The residence time

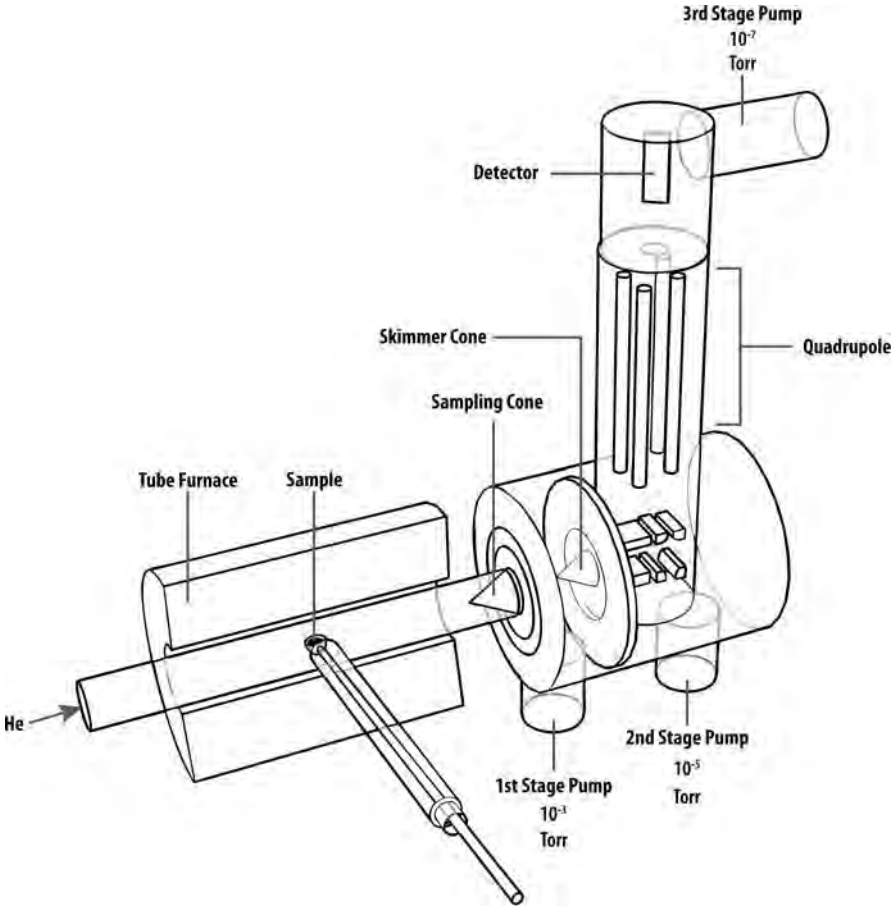

Fig. 1 Experimental schematic of molecular beam mass spectrometer and tube furnace consisting of a quartz tube (2.5 cm inside diameter) with helium flowing through at 5 L/min (at STP)

of the pyrolysis vapors in the reactor pyrolysis zone has been estimated to be ~75 ms and is short enough that secondary cracking reactions in the quartz reactor are minimal. Mass spectral data from *m/z* 20–450 were acquired on a Merlin data acquisition system using 22.5 eV electron ionization. By means of this system, both light gases and heavy tars are sampled simultaneously and in real time. The mass spectrum of the pyrolysis vapor provides a rapid, semi-quantitative depiction of the molecular fragments.

Lignin estimation

The intensities of the major peaks assigned to lignin were summed in order to estimate the lignin contents across the range of samples. Lignin peaks with *m/z* 120, 124, 137, 138, 150, 152, 154, 164, 167, 178, 180, 181, 182, 194, and 210 were

summed and then averaged for the different samples (Table 1). These estimated values were used to determine the variability among the different height and rings of the individual samples. Further chemical composition of masses can be found in Evans and Milne (1987). Syringyl to guaiacol (S:G) ratios were determined by summing the syringyl peaks 154, 167, 168, 182, 194, 208, and 210 and dividing by the sum of guaiacol peaks 124, 137, 138, 150, 164, and 178 (Table 1). Several lignin peaks were omitted in the syringyl or guaiacol summations due to individual peaks having associations with both S and G precursors.

Lignin values estimated by pyMBMS in the study were corrected to approximate Klason lignin values by the following procedure. Klason lignin values were determined for a National Institute of Standards and Technology sample (NIST 8492) by means of standard laboratory procedures (Browning 1967). Six pyMBMS spectra of NIST 8492 were averaged and lignin was estimated by summing the peaks in the previous paragraph. A correction factor was then determined by dividing the Klason lignin value by the lignin value determined by pyMBMS. This

Table 1 Mass spectrum peak assignments associated with pyrolysis molecular beam mass spectrometry for *Populus* wood samples (based on Evans and Milne 1987)

m/z	Assignment	S or G precursor
57, 73, 85, 96, 114	C5 sugars	
57, 60, 73, 98, 126, 144	C6 sugars	
94	Phenol	
110	Catechol, resorcinol	
120	Vinylphenol	
122	Ethylphenol	
124	Guaiacol	G
137[a]	Ethylguaiacol, homovanillin, coniferyl alcohol	G
138	Methylguaiacol	G
150	Vinylguaiacol	G
152	4-ethylguaiacol, vanillin	G
154	Syringol	S
164	Allyl ± propenyl guaiacol	G
167[a]	Ethylsyringol, syringylacetone, propiosyringone	S
168	4-Methyl-2, 6-dimethoxyphenol	S
178	Coniferyl aldehyde	G
180	Coniferyl alcohol, syringylethene	S, G
182	Syringaldehyde	S
194	4-Propenylsyringol	S
208	Sinapyl aldehyde	S
210	Sinapyl alcohol	S

m/z mass:charge ratio of fragments extracted. Major lignin peak assignments and Syringyl (S) or Guaiacol (G) designations

[a] Fragment ion

correction factor was then used to correct the remaining samples to values that are comparable to Klason lignin values.

Experimental reproducibility and quality assurance

Standards, from aspen (*Populus tremuloides*) and a loblolly pine (*Pinus taeda*), known to represent differences in S:G ratio and lignin content, were analyzed along with the ring samples. These internal standards were analyzed at the start and end of each day and periodically throughout the run to monitor spectrometer drift and to identify if other instrumental problems occurred during the analysis. The standard runs in all experiments had excellent reproducibility and show little to no drift. Pooled standard deviations (s_p) for the duplicates in each pyMBMS run were calculated using the formula:

$$s_p = \left[\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \cdots (n_k - 1)s_k^2}{n_1 + n_2 + \cdots n_k - k}\right]^{1/2} \quad (1)$$

where, 1, 2, … k refers to the different series of measurements, s_k is the standard deviation for each set of measurements, and n_k is the number of measurements in each series (McNaught and Wilkinson 1997).

Data analysis

Data analysis was performed using the Unscrambler version 9.1 software program (CAMO A/S, Trondheim, Norway). Each spectrum was averaged and the background was removed by means of Merlin software. The raw data was then imported into Microsoft Excel and formatted for analysis. The Unscrambler software was used to normalize the data based on total ion content to eliminate differences due to variation in sample weights. Principal component (PC) analysis was performed to determine if the samples have different patterns of variation separating them into distinct groups and to provide loading coefficients for the PCs. Coefficients of variation were calculated with the following formula to compare the variability between the heights and rings:

$$C_v = \frac{\sigma}{|\mu|} \quad (2)$$

where σ is the standard deviation and μ is the mean (Rao 1998).

Results and discussion

The data for height and ring properties are presented in Tables 2 and 3. Average lignin content of each tree is shown with coefficients of variation depicting the variability height obtained or ring obtained samples (Table 2). In Fig. 2a the PC scores plots for ring samples can be found. There was no significant height effect for lignin and there was no apparent clustering of samples or pattern to the variation on

Table 2 Descriptive statistics for lignin content of wood disks taken (a) at different heights (0.3–2.4 m) from the base of the tree and (b) from ring samples (1–7) going from pith to bark

Tree	Diameter at 1.2 m	Average lignin (%)	Standard deviation	Range		Number of disks	Coefficient of variation
				Minimum	Maximum		
(a) From different heights (0.3–2.4 m) going up the tree							
1890	2.50″	23.17	0.65	22.69	23.91	3	2.82
1093	3.25″	24.17	0.24	24.00	24.33	2	0.98
1086	3.88″	22.45	0.84	21.67	23.63	4	3.72
1062	7.25″	23.38	1.47	21.90	24.85	3	6.31
129	7.50″	24.75	1.60	22.58	26.33	4	6.48
1592	7.75″	21.80	0.92	21.05	23.05	4	4.22
242	9.50″	23.41	0.37	22.96	23.77	4	1.59
(b) From ring samples (1–7) going from pith to bark							
1890	2.50″	23.56	1.62	20.97	25.15	5	6.89
1093	3.25″	26.01	1.35	24.46	27.66	6	5.18
1086	3.88″	24.68	1.10	22.93	25.97	7	4.47
1062	7.25″	23.61	1.54	20.99	25.59	7	6.52
129	7.50″	26.14	1.46	24.11	27.70	7	5.57
1592	7.75″	24.60	1.93	22.73	27.81	7	7.85
242	9.50″	24.08	1.35	23.06	26.93	7	5.62

Data is sorted by diameter

the PC scores plot (data not shown). Figure 2a does show a decrease in lignin as the number of rings from the pith increases from 1 to 7. It is also apparent that Rings 1 and 2 separate from the remaining five rings, indicating that there was a significant ($P < 0.001$) ring effect on lignin content (Fig. 1a).

Variation of cell wall chemical properties as a function of height

The sampling height at which a wood disk was sampled on the tree did not have a significant effect on the estimate of lignin content as shown by the data in Table 2. The largest change in lignin values was for tree 129 ranging from 22.6 to 26.3%. This 3.7% difference represented the entire range of variation among all sampled heights and trees. In addition, the pooled standard deviation calculated using Eq. (1) of each set of duplicates was less than one percent for lignin. Coefficients of variation calculated from Eq. (2) and ranges of lignin content were not correlated ($r = 0.08$) among large or small trees indicating that the diameter effect on the lignin values was negligible (Table 2a). The S:G ratios estimated from the pyMBMS also did not vary significantly with height up the stem (data not shown).

The majority of the research reported to date on wood properties measured at different heights has focused on physical wood properties. Raymond et al. (1998)

Table 3 Lignin content and Syringyl to Guaiacol ratios (S:G) of individual rings measured from the pith to the bark (i.e., 129-4 represents a wood disk sampled from tree 129 at a height of 1.2 m)

Sample	Lignin content (%)	S:G	Sample	Lignin content (%)	S:G
129-4 R1	27.70	1.6	1093-4 R1	27.66	1.6
129-4 R2	27.49	1.8	1093-4 R2	27.45	1.9
129-4 R3	26.52	2.0	1093-4 R3	24.65	2.3
129-4 R4	27.22	2.0	1093-4 R4	24.46	2.4
129-4 R5	25.30	1.9	1093-4 R5	25.93	2.7
129-4 R6	24.11	2.1	1093-4 R6	25.88	2.4
129-4 R7	24.62	2.0	1592-4 R1	26.41	1.6
242-4 R1	26.93	1.5	1592-4 R2	27.81	1.8
242-4 R2	23.06	1.9	1592-4 R3	24.94	1.9
242-4 R3	24.54	1.9	1592-4 R4	23.19	2.1
242-4 R4	23.87	2.1	1592-4 R5	24.33	2.2
242-4 R5	23.30	1.9	1592-4 R6	22.73	2.3
242-4 R6	23.20	2.0	1592-4 R7	22.83	2.3
242-4 R7	23.64	1.8	1890-4 R1	25.15	1.7
1062-4 R1	23.96	1.4	1890-4 R2	24.62	1.9
1062-4 R2	22.16	1.7	1890-4 R3	20.97	2.3
1062-4 R3	23.90	1.7	1890-4 R4	23.22	2.3
1062-4 R4	25.59	1.9	1890-4 R5	23.83	2.2
1062-4 R5	24.16	1.9			
1062-4 R6	20.99	2.1			
1062-4 R7	24.54	2.1			
1086-4 R1	25.97	1.6			
1086-4 R2	25.41	1.9			
1086-4 R3	23.95	2.1			
1086-4 R4	24.85	2.1			
1086-4 R5	22.93	2.1			
1086-4 R6	23.99	2.1			
1086-4 R7	25.68	2.1			

found that both fiber length and basic density of *Eucalyptus regnans* changed little over the first 30% of total height, but changed significantly over the remaining height. Jones et al. (2005) found that stiffness and microfibril angle of *P. taeda* stays relatively constant over the first 60% of the total tree height. Raymond et al. (2001) found that predicted pulp yield of *Eucalyptus globulus* remains constant with increasing height over 70% of the total height. Typically, as cellulose content increases predicted pulp yield decreases; the inverse is true for lignin content. The results of Raymond et al. (2001), suggesting minimal change in lignin content as height increases, are supported by the results of this study.

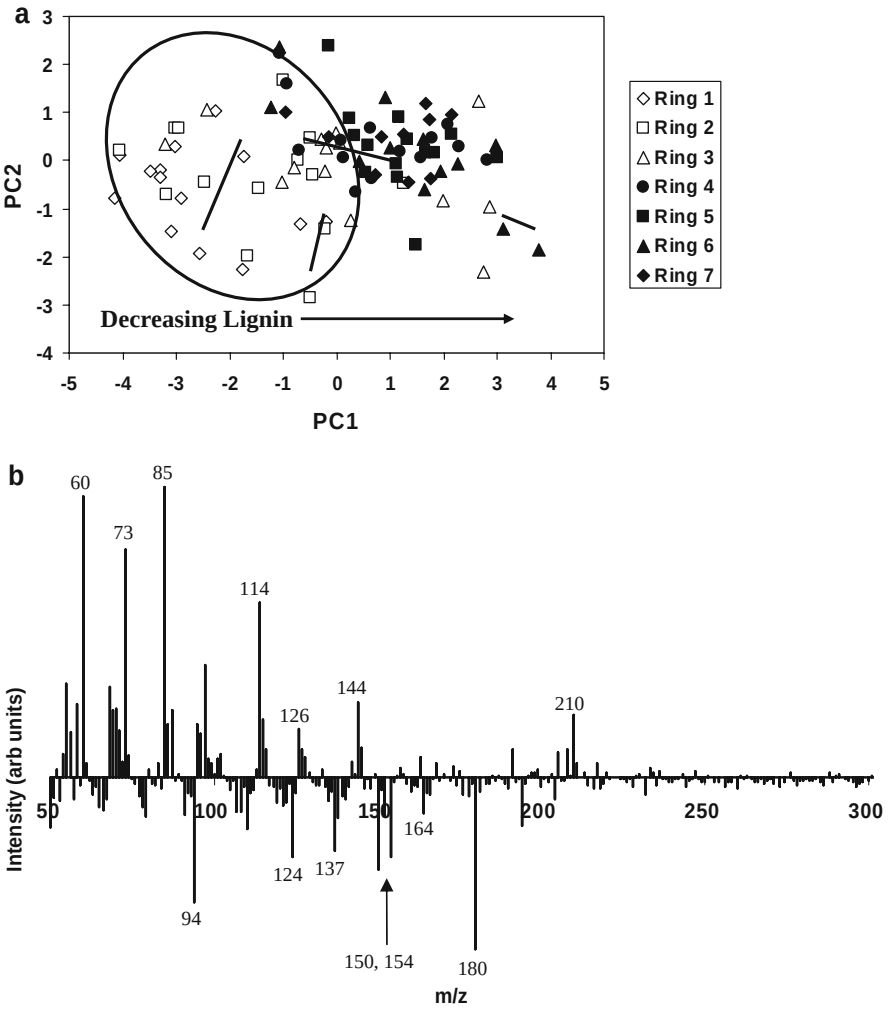

Fig. 2 **a** The principal component 1 (*PC1*) versus principal component 2 (*PC2*) plot of molecular beam mass spectrometry (*MBMS*) data for analyzing individual ring variability. Samples contained inside the oval represent rings 1 and 2. Values connected by *lines* represent technical replicates of the same sample and indicate the typical error of the pyrolysis vapor MBMS analysis. **b** Loading coefficients associated with principal component 1 for individual ring samples of *Populus* trees. Major lignin peaks are shown and assignments are given in Table 1; values above the horizontal line have positive loading coefficients and values below the line have negative coefficients. *m/z* mass:charge ratio of fragments

Variation of cell wall chemical properties as a function of radial position

The scatter plot in Fig. 2a displays the PC1 versus PC2 scores of the mass spectra for all sample rings analyzed by pyMBMS in the mass range *m/z* 50–450. PC1 explains 21% of the variance and is related to lignin content, while 6% of the

variance is explained by PC2 and is attributed to carbohydrate differences. A comparison of the mass spectra for the individual ring samples with positive PC1 loadings and negative PC1 loadings are presented in Fig. 2b. The loading coefficients also contain a series of positive peaks including *m/z* 60, 73, 85, 114, 126, and 144 which all relate to carbohydrate content, while the prominent negative peaks are *m/z* 94, 124, 137, 150, 154, 164 and 180 contributed to phenolics and lignin content (Table 1). The loading coefficients indicate that samples with positive PC1 scores have lower lignin values than samples with negative PC1 scores.

The ring position within a tree had a significant effect on the lignin content ($P < 0.001$). Rings 1 and 2 from the pith outward had negative PC scores, higher lignin content and separated from Rings 3–7 which in general had positive PC scores and lower lignin content (Fig. 1a). Lignin content from Rings 1 to 7 ranged between 20.9 and 27.8%. Although there appears to be minor differences in the overall lignin content across heights and rings, measuring over different rings resulted in larger coefficients of variation. This indicates that when estimating wood properties among *Populus* trees, the ring or rings chosen will have a larger effect on wood properties than the height at which the sample was taken. Similar to the different heights, ranges of lignin content and coefficients of variation were significantly correlated ($r = -0.44$) across the sampled diameters, though there was a slight negative trend with diameter (Table 2b).

The S:G ratios estimated from the pyMBMS showed a slight increase as the number of rings increased from the pith (Table 3). Pilate et al. (2002) estimated S:G of *Populus* using thioacidolysis and found no differences in S:G measured at 6, 24, and 48 months. Pilate et al. (2002) measured S:G from a pooled sample of the stem and not by individual rings, therefore, age differences in S:G would be muted by averaging over all the rings at each age. Pilate et al. (2002) found essentially no change in Klason lignin from 6 to 48 months using pooled samples even though overall lignin content generally decreased as the number of rings from the pith increased.

Earlier studies reported that sinapyl alcohol is released early on in the pyrolysis process indicating that there may be a preferential pyrolysis mechanistic pathway to its formation (Evans et al. 1986; Sarkanen and Ludwig 1971). One possible explanation is that the sinapyl alcohol is released from less-condensed lignin or from lignin structures predominately linked through weaker bonds, such as *β-O*-4 linkages. This may indicate that lignin with higher S:G ratios observed in the more juvenile wood is less condensed than the lignin formed at later stages of tree development. The preferential release of syringyl units in hardwoods could lead to the S/G ratios presented in this paper being overestimated. However, comparisons of with thioacidolysis values performed in our laboratory indicate that the preferential release of syringyl units during pyrolysis is similar to what is observed during thioacidolysis.

It is possible that the differences in wood chemistry between Rings 1–2 and Rings 3–7 are related to juvenile wood formation, although differences between juvenile and mature wood in hardwoods are not pronounced in softwoods (Jett and Zobel 1975). Potentially, there could be differences between the rings if the trees

were differentially subjected to stress such as wind or ice damage at a young age causing tension wood formation. Tension wood composition includes a high crystalline cellulose and low lignin content (Jett and Zobel 1975). It is also possible that there are minor differences in heartwood formation among the sampled trees.

The pooled standard deviation of each set of duplicates calculated from Eq. (1) was 0.67% for lignin content (Fig. 1a). The rings were analyzed at two heights (0.3 and 1.8 m) and did not reveal a significant effect on the wood properties confirming both the individual ring results and that individual rings vary more than different heights.

Conclusion

The height at which a tree was sampled did not have a significant effect on lignin content or S:G ratios. There was, however, a large amount of variation from the pith to the bark suggesting that if one ring is used for wood property assessment, it is imperative that ring selection is carefully considered. Alternatively, a representative, amalgamated sample encompassing all growth rings could prove to be more accurate. Variation in diameter was not correlated with variation in lignin content.

Although only seven trees were sampled in this study, Muneri and Raymond (2001) suggest that sampling 6–8 "whole" trees will result in an accurate measure of wood properties. Caution is still required upon examining the results in this study as chemical wood properties can be substantially affected by the environment in which the trees are grown. Repeating this experiment on a larger and well balanced dataset would further solidify the results. It would also be interesting to look at different species to see whether different species show significant differences in lignin content, as well as which individual ring correlates best with the average of each wood disk.

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