# BETWEEN-TREE VARIATION IN LIGNIN CONCENTRATION IN PINUS RADIATA TRACHEIDS WITH GROWTH RATE, STEM ECCENTRICITY, SITE, AND SILVICULTURAL TREATMENT

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ABSTRACT

Lignin concentration in the tracheid cell wall of **Pinus radiata** D. Don was examined in relation to growth rate, stem eccentricity, site, and silvicultural treatment, using interference microscopy. The greatest variation occurred in the cell corner middle lamella, with a range of values from 76% to 92% v/v. S2 lignin concentration varied from 20% to 22% v/v. The observed variation appears to be independent of any of the factors examined.

**Keywords:** lignin; cell wall layers; tracheids; interference microscopy; **Pinus radiata**.

## INTRODUCTION

Several studies have been made of between-tree variation in the chemical composition of wood. Nylinder & Hägglund (1954) found that geographical location influences cellulose yield, smaller yields being associated with increasing latitude and altitude. Schütt (1958) found that differences between trees of lodgepole pine appeared to depend on provenance rather than site or climatic differences. Zobel & McElwee (1958) found large between-tree differences in cellulose yield, but only small variation with geographic location. Kennedy & Jaworsky (1960) attributed between-tree differences in cellulose content in Douglas fir to genetic effects. Crown class, site, radial position, growth rate, and the proportion of summerwood failed to account for the observed variation in cellulose content. Zobel *et al.* (1960) failed to find any geographic pattern in cellulose yield. Dadswell *et al.* (1961) and Zobel *et al.* (1966) both concluded that the degree of inheritance for cellulose yield was too small to justify selection for improved yield. Elder & Burkart (1983) compared the chemical composition of two ecotypes of loblolly pine and suggested that differences in holocellulose yields might represent adaptations to drought conditions.

Uprichard (1971) examined the within- and between-tree variation in chemical composition of *P. radiata* wood. He found very little variation among trees in cellulose content, appreciable within-tree variation, and a slightly greater variation in lignin content. A slight reduction in cellulose content was observed in a group of trees after fertiliser application. Between-tree variation was assumed to relate to crown develop-

ment. Wilcox (1973) found variation in lignin content (absorption coefficient) among clones of loblolly pine, but it is unclear whether or not this reflected variation in lignin concentration because there was compression wood in the specimens he examined.

The present investigation examined between-tree variation in the quantitative distribution of lignin within the layers of the tracheid cell wall in relation to growth rate, stem eccentricity, site, and silvicultural treatment.

## MATERIALS AND METHODS

Samples were collected as butt discs from 10 *P. radiata* trees in the North Island of New Zealand. Four trees were sampled from Kaingaroa State Forest and three from each of Karioi and Rotoehu State Forests. Growth rate was measured as the average yearly increment along the line of maximum stem diameter on the side opposite to compression wood in eccentric stems. Eccentricity was determined as the ratio of maximum and minimum radii along the line of maximum stem diameter.

Specimens 1 and 2 from Kaingaroa had not been subjected to thinning or pruning, while Specimens 3 and 4 had received such treatment several seasons prior to the growth ring sampled. Trees from Karioi had been subjected to thinning and pruning several seasons prior to the growth ring sampled, while trees from Rotoehu were being thinned at the time of sampling.

Specimens were prepared for interference microscopy as follows. Earlywood from the third growth ring (counting from the bark) of each disc taken from the side opposite to compression wood in eccentric stems, was boiled in water for 6 h followed by extraction with benzene/ethanol 2 : 1 v/v for 3 days, to remove extractives. Specimens were washed sequentially in ethanol and in acetone before being embedded in Spurrs resin. Matched samples of holocellulose were also prepared by treating whole-wood with a mixture of glacial acetic acid and hydrogen peroxide 1 : 1 v/v for 6 to 8 h. Specimens were washed in water, dehydrated in an acetone series, and embedded in Spurrs resin.

Specimens were sectioned in the transverse plane at a thickness of 2 $\mu$m with a glass knife using an LKB ultramicrotome. Sections were treated with sodium ethoxide for 5 min at room temperature to remove the embedding plastic. Refractive index measurements were made in the S2 and cell corner middle lamella (ccml) regions of the tracheid wall. Ethanol and glycerol were used as mounting media. All measurements were made at 20°C at a wavelength of 546.1 nm. Measurements were made with 10 repeats on each of five microscope slides. Refractive indices were converted to lignin concentration values using appropriate calibration values (Boutelje 1972; Donaldson 1985a).

## RESULTS

The variation in S2 lignin concentration of earlywood with growth rate, eccentricity, and site is shown in Table 1, with a corresponding analysis of variance in Table 2. These results indicate no significant variation among sites but there was significant variation among trees within sites. Lignin concentration was not significantly correlated with either growth rate ($r = -0.06$; ns), or eccentricity ($r = 0.13$; ns). In the ccml region there was no significant variation among sites, but there was very highly

significant variation among trees within sites (Tables 1 and 2). Lignin concentration was not significantly correlated with either growth rate ($r = 0.50$; ns), or eccentricity ($r = -0.39$; ns).

TABLE 1—Variation in S2 and ccml lignin concentration with growth rate, eccentricity, and site

| Site | Tree | Growth rate (mm/yr) | Eccentricity | Lignin (%) | |
|---|---|---|---|---|---|
| | | | | S2 | ccml |
| Kaingaroa | 1 | 3.7 | 1.64 | 20 | 81 |
| | 2 | 5.3 | 1.60 | 20 | 76 |
| | 3 | 9.2 | 1.33 | 20 | 82 |
| | 4 | 11.7 | 1.22 | 21 | 85 |
| Karioi | 1 | 8.8 | 2.02 | 22 | 83 |
| | 2 | 10.9 | 1.62 | 21 | 86 |
| | 3 | 15.6 | 1.02 | 20 | 83 |
| Rotoehu | 1 | 5.3 | 1.15 | 22 | 78 |
| | 2 | 8.3 | 1.08 | 21 | 92 |
| | 3 | 12.1 | 1.02 | 21 | 91 |

TABLE 2—Analyses of variance for S2 and ccml lignin concentrations for trees within and between sites

| Source | df | MS | F | MS | F |
|---|---|---|---|---|---|
| Among sites | 2 | 6.22 | 2.8 ns | 144.35 | 1.2 ns |
| Within sites | 7 | 2.25 | 3.4 ** | 124.92 | 208.2 *** |
| Residual | 40 | 0.66 | | 0.60 | |

There was no significant variation in S2 lignin concentration between trees which had been thinned and pruned and those which had not, although there was significant variation among trees within each treatment (Tables 3 and 4). The results for the ccml region were similar to those for the S2 region, although the variation among trees within treatments was much greater.

TABLE 3—Variation in S2 and ccml lignin concentration with presence or absence of silviculture (thinning and pruning)

| S2 lignin (%) | | ccml lignin (%) | |
|---|---|---|---|
| With silviculture | Without silviculture | With silviculture | Without silviculture |
| 20 | 20 | 82 | 81 |
| 21 | 20 | 85 | 76 |
| 22 | 22 | 83 | 78 |
| 21 | 21 | 86 | 92 |
| 20 | 21 | 83 | 91 |

TABLE 4—Analyses of variance for S2 and ccml lignin concentration with presence or absence of silviculture

| Source | df | MS | F | MS | F |
|---|---|---|---|---|---|
| Among treatments | 1 | 0.01 | 0.0 ns | 0.32 | 0.0 ns |
| Among trees | 8 | 3.52 | 5.4 *** | 145.35 | 242.3 *** |
| Residual | 40 | 0.66 | | 0.60 | |

## DISCUSSION

The results of these experiments indicate that variation in lignin concentration in the tracheid cell wall does not relate directly to growth rate, eccentricity, site, or the presence or absence of thinning and pruning treatments in the trees examined.

Although variation in lignin concentration in the S2 region is small enough to be considered negligible, variation in the ccml region covers a range of 16% which is too large to ignore. It has already been shown that this variation is not related to position within the growth ring or to radial position within the stem. The variation is known to be consistent within the tree (Donaldson 1985b). The range of variation is similar to that found in a previous study where values ranged from 72% to 92% (Donaldson 1985b). Boutelje (1972) reported a similar range for lignin concentration in *Pinus sylvestris* L., although apparently the range he gave applied to earlywood and latewood from a single tree.

The absence of any correlation between lignin concentration in normal wood and the eccentricity of the stem supports the conclusions of Larson (1966) and Timell (1973a, b) that opposite wood has the same chemical composition as normal or side wood, and refutes other reports of chemical differences by Dadswell *et al.* (1958) and Larson (1969).

Assuming that the observed variation in ccml lignin concentration is not related to environmental factors, then the variation must be genetically controlled. Examination of clonal material could provide confirmation of this. Trees with a low ccml lignin concentration should be easier to pulp by chemical means than trees with high ccml lignin concentration. Investigations into the heritability of this trait might prove useful from an industrial point of view.

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