WITHIN- AND BETWEEN-TREE VARIATION IN LIGNIN CONCENTRATION IN THE TRACHEID CELL WALL OF PINUS RADIATA

L. A. DONALDSON

Forest Research Institute, New Zealand Forest Service,
Private Bag, Rotorua, New Zealand

(Received for publication 23 December 1985)

ABSTRACT

The variation in lignin concentration between earlywood and latewood, corewood and outerwood, branch wood, root wood, and compression wood was determined for the S2 and cell corner middle lamella regions of the tracheid cell wall of **Pinus radiata** D. Don tracheids, using interference microscopy, The average lignin concentration in the S2 region was 21% v/v while the cell corner middle lamella had a value of 81% v/v. The greatest variation among trees occurred in the cell corner middle lamella region. The lignin concentration of 24% in the S2 region in mild compression wood was slightly higher than normal.

Keywords: lignin; earlywood; latewood; corewood; outerwood; interference microscopy; cell wall layers; **Pinus radiata**.

INTRODUCTION

The chemical composition of coniferous wood varies with position within the tree, both radially and with height. Ritter & Fleck (1926) studied differences in chemical composition between earlywood and latewood in a range of softwoods and hardwoods. These authors found that earlywood contained more lignin and less cellulose than latewood. The difference was explained in terms of the structure of the cell wall. The middle lamella forms a slightly greater part of the total cell wall volume in earlywood than in latewood, and has a higher lignin concentration than the rest of the wall. The difference in wall thickness between earlywood and latewood due to changes in the thickness of the S2 layer has a diluting effect which could account for the change in the cellulose/lignin ratio. Other workers have found similar results (Hale & Clermont 1963; Larson 1966; Siddiqui 1976; von Byrd *et al.* 1965; Watson & Hodder 1954; Wilson & Wellwood 1965; Wu & Wilson 1967). Changes in lignin content from corewood to outerwood have also been related to changes in cell wall morphology (the proportions of different wall layers), with a higher lignin content (lower cellulose content) in corewood than in outerwood (Harwood 1971; Kennedy & Jaworsky 1960; Larson 1966; Uprichard 1971; van Buijtenen *et al.* 1961; von Byrd *et al.* 1965; Zobel & McElwee 1958; Zobel *et al.* 1966).

This report is part of a detailed investigation of the relationship between lignin concentration, cell wall morphology, and lignin content in *P. radiata.*

MATERIALS AND METHODS

Wood specimens were collected as butt discs from five 50-year-old trees of *P. radiata* growing in Kaingaroa State Forest, central North Island, New Zealand. Wholewood samples were collected from the corewood (Growth Ring 49 counting from the bark) and outerwood (Growth Ring 3 from the bark) of each disc. Samples were divided into earlywood and latewood fractions and extracted in benzene/ethanol 2 : 1 v/v for 3 days to remove resinous material. The specimens were dehydrated in acetone and were subsequently embedded in Spurrs resin. Matched earlywood samples were used to prepare holocellulose by treating with glacial acetic acid/hydrogen peroxide 1 : 1 v/v for 6 h at 80°–90°C. Holocellulose material was then dehydrated in an acetone series and embedded in Spurrs resin.

Transverse sections 2 μm in thickness were used for measurement of refractive index according to the method described by Donaldson (1985), using a Zeiss Photomicroscope II equipped for quantitative interference microscopy. Embedding plastic was removed from the sections by treating with sodium ethoxide for 5 min at room temperature. Measurements were made in the S2 and cell corner middle lamella (ccml) regions of wholewood and in the S2 region of holocellulose fibres. Refractive index measurements were then converted to lignin concentration values expressed as percentages on a volume basis (Boutelje 1972; Donaldson 1985). All measurements were made at 20°C at a wavelength of 546.1 nm using ethanol and glycerol as mounting media.

Results were analysed using a mixed model factorial ANOVA with factors labelled as "trees", "age" (corewood *v.* outerwood), and "position" (earlywood *v.* latewood). "Trees" was regarded as a random effect while "age" and "position" were regarded as fixed treatment effects (Sokal & Rohlf 1981). Results for the S2 and ccml regions were analysed separately.

Specimens of branch wood, root wood, and compression wood were collected from trees of *P. radiata* growing in Rotoehu State Forest, Bay of Plenty, New Zealand. Single specimens of earlywood were prepared and analysed in a similar fashion to that described above.

RESULTS

The results for the S2 region are shown in Table 1 and an analysis of variance in Table 2. There is significant random variation among trees. There are also significant two-way interactions between trees and age, and trees and position, and a significant three-way interaction between trees, age, and position. Only random variation is present, indicating no consistent variation between earlywood and latewood or between corewood and outerwood. The within-subgroups variance accounts for 47% of the total, the main effect of trees accounts for 9%, the two-way interaction between trees and age for 11%, the two-way interaction between trees and position for 9%, and the three-way interaction between trees, age, and position for the remaining 24%. The average lignin concentration in the S2 region is 21% v/v with a between-tree range of 20–22% and

TABLE 1—Variation in S2 lignin concentration (%) within and between trees

Treatment	Tree					Treatment means
	1	2	3	4	5	
Corewood/earlywood	22	21	21	20	22	21
Corewood/latewood	22	23	21	20	20	21
Outerwood/earlywood	22	19	22	20	20	21
Outerwood/latewood	21	21	22	20	20	21
Tree means	22	21	22	20	21	21

TABLE 2—Analysis of variance of S2 lignin concentration within and between trees

Source	df	SS	MS	F
A	4	38.09	9.52	5.0 **
B	1	3.17	3.17	0.5 ns
AB	4	25.17	6.29	3.3 *
C	1	0.01	0.01	0.0 ns
AC	4	21.65	5.41	2.8 *
BC	1	0.85	0.85	0.1 ns
ABC	4	27.52	6.88	3.6 *
R	80	152.00	1.90	

A = Trees, B = Age (corewood **v.** outerwood), C = Position (earlywood **v.** latewood), R = Residual

an over-all range of 19–23%. The branch wood earlywood and root wood earlywood samples examined had values of 22% and 21% respectively and are within the range shown by normal stem wood. Compression wood, which had a value of 24%, was just outside this range.

The results for the ccml region are shown in Table 3 and an analysis of variance in Table 4. There is very highly significant random variation between trees which accounts for 38% of the total random variation. The remaining 62% corresponds to the residual variance component. The average lignin concentration in the ccml region is 81% v/v with a between-tree range of 74–88% and an over-all range of 72–92%. The ccml region of branch wood, root wood, and compression wood cell walls had lignin concentrations of 77%, 78%, and 89% respectively.

DISCUSSION

There were no consistent differences for S2 lignin concentration between earlywood and latewood or between corewood and outerwood. It seems likely that the small variation that did occur was due to experimental error. For example, Donaldson (1985) has predicted an error of ± 2% from differences in orientation between wholewood

TABLE 3—Variation in ccml lignin concentration (%) within and between trees

Treatment	Tree					Treatment means
	1	2	3	4	5	
Corewood/earlywood	87	72	83	79	92	83
Corewood/latewood	79	75	80	80	89	81
Outerwood/earlywood	84	74	85	82	84	82
Outerwood/latewood	77	76	75	78	85	78
Tree means	82	74	81	80	88	81

TABLE 4—Analysis of variance of ccml lignin concentration within and between trees

Source	df	SS	MS	F
A	4	1881.44	470.36	13.4 ***
B	1	77.44	77.44	1.7 ns
AB	4	178.76	44.69	1.3 ns
C	1	174.24	174.24	2.0 ns
AC	4	343.96	85.99	2.4 ns
BC	1	14.44	14.44	0.4 ns
ABC	4	92.16	23.04	0.7 ns
R	80	2815.24	35.19	

A = Trees, B = Age (corewood **v.** outerwood), C = Position (earlywood **v.** latewood), R = Residual

and holocellulose specimens. Except for compression wood, all of the specimens examined showed values restricted to within ± 2% from the mean. It is worth noting that this compression wood specimen was very mild as judged by the absence of helical fissures in the secondary wall even though inter-cellular spaces were abundant. The technique seems to be sufficiently sensitive to detect small differences in lignin concentration as might be expected in a mild compression wood specimen.

The absence of helical fissures in a compression wood specimen with only a slight increase in lignin concentration supports the theory of Boyd (1973) who suggested that lignification results in swelling of the cell wall which in turn causes the formation of helical fissures. If the degree of lignification is close to normal then helical fissures should be poorly developed or absent.

Branch wood and root wood had a similar S2 lignin concentration to normal wood. It is interesting to note that the root wood specimen did not show any of the features of compression wood in spite of its extremely eccentric growth. The absence of compression wood in eccentric root material has been noted by several workers (Westing 1965; Patel 1971; Donaldson 1983).

Boutelje (1972) studied the S2 lignin concentration of *Picea abies* Karst. and *Pinus sylvestris* L. using interference microscopy and found values between 18% and 22% v/v. These values are similar to values found for *P. radiata* in the present study.

Fergus *et al.* (1969) used UV microscopy to determine lignin distribution in wood of *Picea mariana* (Mill.) B.S.P. and found values of 22.5% and 22.2% w/w in the secondary wall of earlywood and latewood tracheids respectively. The volume concentration found in the present study can be converted to a weight concentration using the densities of lignin and holocellulose. Assuming these values to be 1.335 and 1.521 respectively (Stamm & Sanders 1966), this yields an average value of 18% which is lower than the values given by Fergus *et al.* (1969). The difference in lignin concentration between earlywood and latewood reported by these authors is even smaller than that given in Table 1 and can be regarded as negligible.

Wood & Goring (1971) studied lignin concentration in the stem and branch wood of *Pseudotsuga menziesii* (Mirb.) Franco using UV microscopy. These workers found concentration values of 24.8% and 22.8% respectively. These values are higher than those found in the present work on *P. radiata.* In branch wood which contained severe compression wood, values of 36.3% for the S2 layer and 54% for the S2 (L) layer were reported by these authors. These values are much higher than those for the compression wood examined in the present study although this could be expected because of the greater severity of the compression wood examined by these authors.

Whiting *et al.* (1981) and Whiting & Goring (1982) found values of 21% and 22% lignin w/w by analysing secondary wall fragments. The difference between these values and the value of 18% found in the present report can be explained by the presence of S3 material in the fraction analysed by these workers. The S3 layer of spruce tracheids often has a higher lignin concentration than the S2 layer (Jayme & Fengel 1961; Sachs *et al.* 1963; Scott & Goring 1970). This would have the effect of increasing the lignin content of the fraction analysed by a small percentage. A similar explanation applies to the results of Sorvari *et al.* (1983).

While there are no "significant" differences in ccml lignin concentration between earlywood and latewood or corewood and outerwood, there does seem to be a reasonably consistent pattern of variation between earlywood and latewood. Earlywood tends to have a slightly higher lignin concentration than latewood and this is more pronounced in the outerwood than in the corewood. This trend seems to be related to the thickness of the secondary wall (latewood has thicker walls in the outerwood), and could be interpreted as an effect of secondary wall thickness on the rate of lignification in the ccml region. Although lignification of the ccml region starts before secondary wall formation begins, lignification probably continues in this region until cell wall formation is complete (Wardrop 1981). The secondary wall could therefore restrict movement of enzymes (Cowling 1975; Reese 1977) or lignin precursors into the ccml region. The relative times of the onset of lignification in the ccml region and the start of secondary wall formation could therefore account for some of the variability of lignin concentration in the ccml region, including the prominent between-tree variation. Further examination of the variation between earlywood and latewood with greater replication could yield interesting results.

The between-tree variation in ccml lignin concentration described in this report is too large to be attributed to experimental error. Donaldson (1985) has suggested that measurements of lignin concentration in the ccml region should be more accurate than those in the secondary wall because of the absence of error due to differences in

orientation among samples. In addition, variation in the carbohydrate component of the ccml region should only account for a maximum of about 25% of the observed variation in refractive index.

Boutelje (1972) found the lignin concentration in the ccml region of *Picea abies* and *Pinus sylvestris* tracheids to be within the range 73% to 87% v/v using interference microscopy. Other workers using UV microscopy and micro-chemical analysis have found values ranging from 38% to 100% w/w. On a weight basis the result for *P. radiata* is 71% which agrees with the value of 71% found by Bailey (1936) using micro-chemical analysis of middle lamella fragments from *Pseudotsuga menziesii* wood. These and other results are summarised in Table 5.

TABLE 5—Summary of lignin concentration (%) in the layers of the cell wall determined by various techniques

Genus	Method	S2	cml	ccml	Reference
Picea	IM	Ew 18 Lw 16		74 74	Boutelje (1972)
Pinus	IM	Ew Lw 19		64 77	Boutelje (1972)
Picea	UV	Ew 23 Lw 22	50 60	85 100	Fergus **et al.** (1969)
Pseudotsuga	UV	Ew 25 Lw 23	56 60	83 88	Wood & Goring (1971)
Abies	UV	Ew 25–26 Lw 21–27	46–48 39–52		Fukazawa & Imagawa (1981)
Picea	UV		38–79		Boutelje & Eriksson (1982)
Picea	UV	Ew 23–24 Lw 22–26	48–53 49–53		Takano **et al.** (1983)
Picea	UV	25	48		Boutelje & Eriksson (1984)
Pseudotsuga	Micro		71		Bailey (1936)
Picea	Micro		52–65		Iwamida **et al.** (1975)
Picea	Micro	21	52		Whiting **et al.** (1981)
Picea	Micro	22	60		Whiting & Goring (1982)
Picea	Micro	25 26	50 39		Sorvari **et al.** (1983)
Picea	Micro		40		Westermark (1985)

IM = Interference microscopy
UV = Ultraviolet microscopy
Micro = micro-chemical analysis

Ew = Earlywood
Lw = Latewood
cml = compound middle lamella
ccml = cell corner middle lamella

Values for branch wood, root wood, and compression wood are all within the range shown by normal stem wood. The result for compression wood contrasts with that for the secondary wall where lignin concentration was outside the normal range. Apparently the increase in lignin content of compression wood is due primarily to an increase in lignin concentration in the secondary wall (Timell 1981). The presence of intercellular spaces will of course reduce the contribution of the ccml region to the over-all lignin content of compression wood.

CONCLUSIONS

The results of this investigation indicate little if any variation in the lignin concentration of the S2 region apart from compression wood. In contrast, the ccml region shows significant variation among trees and there is some indication of differences between earlywood and latewood.

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