BORON DEFICIENCY AND TRACHEID PROPERTIES OF PINUS RADIATA

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ABSTRACT

The effect of boron (B) nutrition on some wood properties of *Pinus radiata* D.Don was assessed on 8-year-old trees from a boron fertiliser study at Ashley Forest. Wood samples were taken at breast height (1.4 m). There were significant differences between samples from control trees and those treated with boron fertiliser, with regard to lumen diameter and cell wall thickness. However, differences in tracheid length were only marginal. Cell wall thickness in the samples from boron-treated trees was greater by 35% in earlywood and 25% in latewood. Cell lumen diameter was greater by 29% in earlywood and 46% in latewood. From these results it was evident that application of boron fertiliser produced an increase in both tracheid cross-sectional area and wall thickness. Furthermore, during microtomy the sections from the control samples tended to check radially, indicating possible differences between control and boron-treated trees in the microstructure and composition of wood cell walls. The staining of control sections with toluidine blue and phloroglucinol was consistently less intense than in the sections from boron-treated trees, and the differences in the staining intensity observed were pronounced enough to suggest that boron may have an effect on the lignification of wood cell walls.

Keywords: boron; wood cell walls; *Pinus radiata*

INTRODUCTION

Boron deficiency is well-recognised in New Zealand pine plantations as it causes terminal bud death and permanent stem malformation (Will 1985; Hunter *et al*. 1987). Circumstantial evidence in New Zealand and Australia also suggests a role for boron in determining wood strength or "brittleness". At Ashley Forest in the Canterbury region pine crops show minimal snow damage where the forest crop has been routinely treated with boron fertiliser compared to non-treated trees (A.McCord, pers. comm.). In the Wairarapa district (south-east of the North Island and exposed to hot drying winds in spring and summer), prior to application of boron fertiliser it was observed that "broken tops" were a

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feature of the crop. After adopting routine application of boron fertiliser as a standard management tool, the incidence of broken tops was all but overcome (D.McIntosh, Putinka Forestry Trust, pers. comm.). In the Australian Capital Territory, Snowden & Stahl (1980) reported that pines adequately nourished with boron were minimally infested by *Dothistroma pini* Hulbary, and that pines with chronic boron-deficiency symptoms were predisposed to infection presumably through anatomical malformations and physiological disturbances. D.Jamieson (pers. comm.) reports a connection between boron deficiency and *Diplodia* infestation at Tumbarumba in New South Wales.

Spurr (1957) reported plant tissue "brittleness" with classical boron deficiency symptoms in contrast to "flexibility" with high boron concentrations. Loomis & Durst (1992) reviewed the chemistry and biology of boron, citing Lewis (1980) and the arguments "that provide strong support for a role for boron in cell wall formation and stability in vascular plants". Skok (1958) reported research showing that absence of boron "may result in various types of distortion, cracking, splitting, or checking".

Recent research has provided evidence for a structural role for boron in the cell wall (Matoh *et al*. 1993; Hu & Brown 1994; Matoh 1997). It is now well known that the cell wall pectic polysaccharide rhamnogalacturonan II (RGII) is covalently linked by a borate ester (O'Neil *et al.* 1996; Ishii & Matsunaga 1996; Kobayashi *et al*. 1996; Ishii *et al.* 2001). Fleisher *et al.* (1999) showed that the dimeric RGII is needed for the formation and stabilisation of the pectin network in plant cell walls. Brown *et al.* (2002) in a review of boron in plant biology concluded that the role of boron may very likely extend past being essential for cell wall structure and function. Recent work by S.Olykan (pers. comm.) showed brittleness of *P. radiata* seedlings grown for 2 months in solution culture where boron solution was maintained at a nominal "zero" concentration (actual concentration was 5 ppb B). Ryden *et al.* (2003) showed that the Arabidopsis mur1 mutant lacking fructose in the RGII resulted in a conformation change, lowering the binding efficiency with boron. Hypercotyl tensile strength and tensile modulus were lowered but the condition could be overcome by adding excess boron.

The work reported here is part of an extended study to investigate the effect of boron deficiency on growth and wood properties of *P. radiata*.

MATERIALS AND METHODS

The Site

The site is located in Ashley Forest in Canterbury (lat.43°11´S, long.172°37´E) and was planted with *P. radiata* in 1982. An experiment to assess the effectiveness of a range of boron fertiliser materials in correcting boron deficiency was established in 1984. The design was a randomised complete block with three replications. At the time of sampling (1990, when the trees were aged 8 years) windthrow had destroyed one of the blocks, leaving only two blocks for sampling. Trees for sampling were from plots receiving ulexite chip (2–5 mm) at 60 kg product/ha. This was seen as the source offering the optimum long-term boron availability. There were two such plots within each block. Foliar boron concentrations in the treated and control plots averaged 17 μg/g and 10 μg/g respectively. At the time of sampling this was the only replicated trial with boron treatments available for sampling.

Sampling

Twelve *P. radiata* trees were felled to investigate their anatomical properties; three trees were selected at random from the control and ulexite-treated plots in each of the two blocks. Discs were cut at breast height on each tree. Sample pieces (1 × 1 × 1 cm) were cut from the outer rings to measure the cell lumen diameter and cell wall thickness of tracheids, and "match-sticks" (2- to 3-cm-long samples) were used for tracheid length measurements from earlywood and latewood of each tree.

Measurements

Sections from the 1 × 1 × 1-cm cubes were cut (30–50 μm thickness) with a sliding microtome, stained with 0.5% toluidine blue solution (prepared in 1% borax) and phloroglucinol, and then mounted in glycerol, for a qualitative indication of lignification.

Thirty-two measurements of radial lumen diameter and radial wall thickness were made on earlywood and latewood tracheids. These measurements were performed on a random selection of sections. The means of each set of 32 measurements were used in subsequent analysis.

The "matchstick" pieces cut from earlywood and latewood were macerated in a 1:1/V:V mixture of glacial acetic acid and hydrogen peroxide at 90–100°C for over 10 hours. After washing, the separated fibres were stained with 1% safranin solution, and mounted in glycerol. Fifty measurements of tracheid length were made using a Leitz digital-measuring wheel, on a random selection of the separated fibres. The means of each set of 50 measurements were used in subsequent analysis.

Analysis of variance was carried out on the data using the SAS procedure GLM (SAS Institute 2000).

RESULTS

Tracheid Length, Lumen Diameter, and Wall Thickness

Application of boron fertiliser to *P. radiata* trees at this boron-deficient site resulted in significant changes in important tracheid properties. Results for both earlywood and latewood are given in Table 1. Although tracheid lengths did not differ significantly ($p<0.05$), lumen diameter and cell wall thickness did show significant differences. As a

TABLE 1–The effect of boron fertiliser on earlywood and latewood tracheid dimensions in *P. radiata*

Tracheid properties	Fertiliser boron		Statistical test	
	No	Yes	F ratio	Pr>F
Earlywood				
Tracheid length (mm)	2.67	2.97	1.61	0.332
Lumen diameter (μm)	22.98	29.55	21.56	0.043
Cell wall thickness (μm)	5.59	7.54	6.63	0.123
Latewood				
Tracheid length (mm)	2.11	2.38	7.61	0.110
Lumen diameter (μm)	10.52	15.41	14.62	0.062
Cell wall thickness (μm)	6.73	8.40	29.48	0.032

result of fertiliser application, cell wall thickness increased by 35% in earlywood (p=0.12) and 25% (p=0.03) in latewood; cell lumen diameter increased by 29% in earlywood (p=0.04) and 46% in latewood (p=0.06). These results provide clear evidence for a link between boron and growth of tracheids.

According to the nested analysis of variance (not presented), tracheid dimensions were not affected by the position of the plots within the experiment. Therefore variation across the plots was minimal, and this adds considerable strength to the basing of results on two blocks where the degrees of freedom in the error term were small.

Qualitative Differences in Cell Wall Staining

During microtomy a majority of the sections from the control (low foliar boron) samples tended to check radially, which was an early indication that there might be differences between control and fertiliser-treated samples with respect to the microstructure and composition of wood cell walls. The staining of sections with toluidine blue, which is known to impart green or greenish blue colour to lignified tissues, showed differences between the samples from the control and fertiliser-treated plots in staining intensity (Fig. 1).

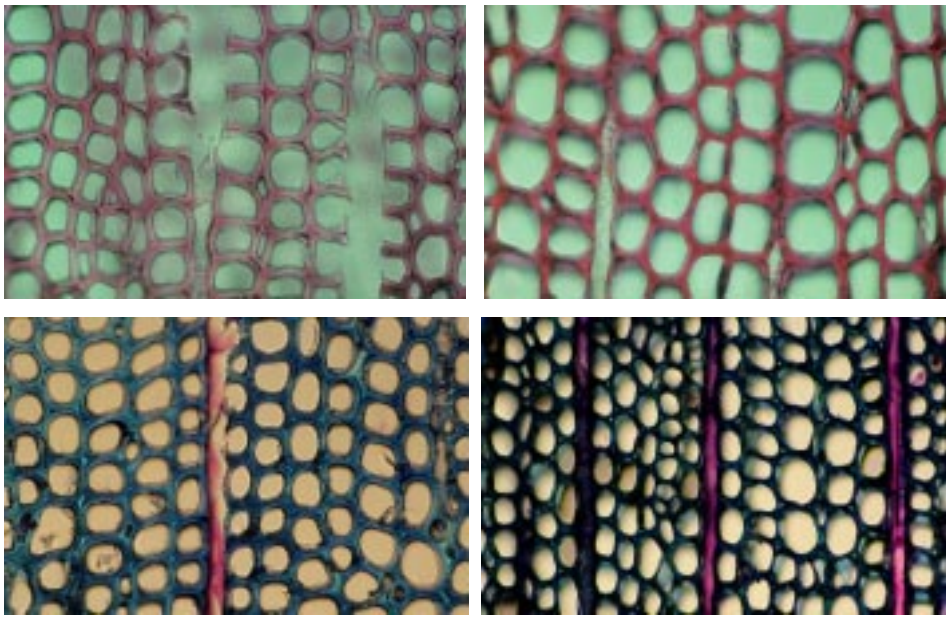

FIG. 1–Photomicrographs showing differences in the appearance of tracheid cross-sections formed under conditions of boron deficiency and boron sufficiency, as revealed with phloroglucinol-HCl and with toluidine blue stain.

(a) Poor and patchy staining with phloroglucinol-HCl for cell wall for lignin, some ray abnormality, and some tracheid wall distortion in the control cross-section compared with the tracheid cross-section from a tree with boron fertiliser applied. This cross-section shows more intense and uniform staining of cell walls.

(b) Staining with toluidine blue shows reduced staining of the cell wall for lignin in the control cross-section which is most apparent at the ray-axial tracheid juncture.

The staining of control sections was consistently less intense than that of the tracheid walls in the sections from fertiliser-treated trees and showed two discrete populations of visible colour difference. Although the staining reaction provided only a qualitative indication, the differences in the staining intensity observed were pronounced enough to suggest that boron may have an effect on the lignification of wood cell walls. A difference in the staining intensity was similarly observed after staining with phloroglucinol-HCl (not illustrated here).

DISCUSSION

At the Canterbury site where the trees used in this study had been planted, summer droughts are common and boron is applied routinely to augment the low boron-supplying capacity of the natural soil. Our investigation of the effect of boron nutrition on wood properties under low levels of boron supply in this field study provided evidence for a close relationship between boron nutrition and certain tracheid properties.

Lohnis (1940) (quoted by Spurr 1957) showed that the "initial disturbance of boron deficiency occurs in the cell walls". Lorenz (1942) reported the presence of thin-walled parenchyma cells and a degeneration of the middle lamella where boron nutrition was limiting. Spurr (1957), working with celery, concluded that boron deficiency produces plants with weak cell walls, while boron in excess results in plants with abnormally resilient walls. Weakened cell walls may have been the cause of pine seedlings showing "S" twisting of the stems where boron supply was low (Olykan 1993).

The increase we observed in the thickness of wood cell walls from trees treated with boron fertiliser appears to be anomalous in relation to the often-quoted increase in cell wall thickness with boron deficiency (Lee & Aronoff 1966; Loomis & Durst 1992). In this study the controls were classified as "boron deficient" and the fertiliser-treated trees as "boron sufficient", based on foliar boron concentrations and the occurrence of tip die-back. However, it is important to bear in mind that these "labels" could be misleading in relation to changes we have observed in cell wall thickness. Another possible explanation may be that boron requirements for primary and secondary growth may be different in terms of the quantity of this nutrient required at specific development stages. The pattern of secondary cell wall growth is certainly different from that of primary cell walls.

The increase in cell wall thickness resulting from boron fertiliser application should have implications for structural stability. Olykan *et al.* (1995) showed that boron fertiliser had a significant effect on foliar biomass and on stemwood production at a site comparable to that described for this study. T.Payn (pers. comm.) showed that young *Pseudotsuga menziesii* (Mirb.) Franco treated with boron in the presence of adequate nitrogen and phosphorus showed marked increases in fascicle weight 1 year after application. These observations on biomass increase are consistent with observations of a boron-related increase in tracheid dimensions presented here.

Recent research with other plant species has identified a role for boron in the covalent cross-linking of complex pectic polysaccharides (RGII) by a borate ester. O'Neill *et al.* (1996) concluded that "cell wall-localised, boron-containing pectic polysaccharides are required for the normal growth and development of plants". Harris (2002) discussed how reduced cross-linking of the RGII molecules via 1:2 borate-diol esters will result in reduced

cell wall strength. Additionally, alteration in pectin architecture of the compound middle lamella resulting from boron deficiency is likely to lead to abnormal lignification of this region in the secondary cell wall.

The fact that pines can grow at very low levels of boron supply in the absence of summer drought suggests that drought-induced water restrictions may have an important influence on xylem development, and this effect is likely to be related to the supply of boron in the cambial region. Recent observations by Donaldson (2002), which have provided evidence for a link between abnormal lignin distribution in *Pinus radiata* wood cell walls and drought stress, support this assumption. He showed the presence of concentric shelling in a severely drought-stressed *P. radiata* tree, relating this phenomenon to poor and irregular lignification of the middle lamella. Barnett (1976) concluded that the effect of drought was to halt differentiation of tracheids at a stage prior to lignification. Lewis (1980) proposed that a primary role for boron was in lignin biosynthesis. The collective evidence suggests a close relationship of boron to the growth and architecture of the middle lamella and the primary wall during cell expansion, and to cell wall lignification after the completion of expansion growth. In this study, the correlative increase observed in the lignification of the middle lamella, as defined by differential staining in the wood samples from fertiliser-treated trees, provides further evidence for a role played by boron in tracheid stability. Adequate lignification of the middle lamella is vital for tracheid adhesion and stability. A transmission electron microscope study (A.Singh & L.A.Donaldson unpubl. data) comparing internally checked and non-checked discs from *P. radiata* trees growing in New Zealand showed reduced and irregular lignification of tracheid walls. This feature was particularly pronounced in the regions of cell wall contacts with rays, where internal checking originates caused by collapse of adjoining tracheids.

Normal lignification of both the compound middle lamella and secondary wall regions is important for the strength and stability of wood tissues. Thus there is need to extend this study to more clearly understand the effect of boron on cell wall lignification in forest trees in general and *P. radiata* in particular using experimental approaches where boron levels can be closely monitored.

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