BRILL

IAWA Journal 35 (4), 2014: 385–394

CHARACTERIZING COMPRESSION WOOD FORMED IN RADIATA PINE BRANCHES

Xinguo Li[1,5], **Robert Evans**[2], **Washington Gapare**[1], **Xiaohui Yang**[1,3] and
Harry X. Wu[1,4,5]

[1]CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia
[2]CSIRO Materials Science and Engineering, PO Box 312, Clayton South, Victoria 3169, Australia
[3]Department of Biotechnology, Beijing Forestry University, Beijing 100083, China
[4]Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology,
Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden
[5]Corresponding authors; e-mail: xinguo.li@csiro.au and harry.wu@csiro.au

ABSTRACT

The formation of reaction wood is an adaptive feature of trees in response to various mechanical forces. In gymnosperms, reaction wood consists of compression wood (CW) and opposite wood (OW) that are formed on the underside and upperside of bent trunks and branches. Although reaction wood formed in bent trunks has been extensively investigated, relatively little has been reported from conifer branches. In this study SilviScan® technology was used to characterize radiata pine branches at high resolution. Compared to OW formed in the branches, CW showed greater growth, darker colour, thicker tracheid walls, higher coarseness, larger microfibril angle (MFA), higher wood density, lower extensional stiffness and smaller internal specific surface area. However, tracheids of CW were similar to those of OW in their radial and tangential diameters. These results indicated that gravity influenced tracheid cell division and secondary wall formation but had limited impact on primary wall expansion. Furthermore, seasonal patterns of CW formation were not observed in the branches from cambial age 4 while earlywood and latewood were clearly separated in all rings of OW. The marked change of MFA during reaction wood formation suggested that branches could be ideal materials for further study of cellulose microfibril orientation.

Keywords: Compression wood, tracheid, wood stiffness, microfibril angle, conifers, SilviScan.

INTRODUCTION

Trees adjust their growth orientation in response to various environmental stresses (*i.e.*, wind, snow, light, gravity, artificial bending) and during this process reaction wood is formed (Nicholls 1982; Yamashita *et al.* 2007). In gymnosperms compression wood (CW) and its opposite wood (OW) are formed on the underside and upperside, respectively, of inclined (bent) trunks and naturally growing branches (Timell 1986; Donaldson & Singh 2013). CW is also found in some straight conifer trees (Donaldson *et al.* 2004; Warensjo & Rune 2004) if they have experienced displacement forces

Published by Koninklijke Brill NV, Leiden

DOI 10.1163/22941932-00000073

at younger ages. Juvenile wood formed in the young conifer trees often contains a considerable proportion of CW. For example, 18% CW was found in young loblolly pine trees (Bendtsen 1978) and this proportion can be as high as 44% (Zobel & McElwee 1958). Compared to normal wood (NW, formed in normal conditions) and OW, CW has shorter tracheids with larger microfibril angle (MFA) in the S_2 layer, greater shrinkage potential, higher wood density, lower stiffness, more lignin and lower cellulose content (Timell 1986; Donaldson & Singh 2013). Therefore, CW is generally considered undesirable for both solid wood and pulp products (Timell 1986; Lohrasebi *et al.* 1999; Ban *et al.* 2004; Yeh *et al.* 2005; Akbulut & Ayrilmis 2006).

CW and OW in naturally inclined and artificially bent trees has been extensively characterized in several conifer species, including loblolly pine (Yeh *et al.* 2005; Yeh *et al.* 2006a), radiata pine (Donaldson *et al.* 2004; Kibblewhite *et al.* 2005; Diaz-Vaz *et al.* 2009; Tarmian & Azadfallah 2009; Lachenbruch *et al.* 2010), Norway spruce (Gindl 2002; Brandstrom 2004; Tarmian & Azadfallah 2009), *Larix* (Yoshizawa *et al.* 1987), Japanese cypress (Yamashita *et al.* 2009), and *Cryptomeria japonica* (Yamashita *et al.* 2007). These studies have revealed distinct properties of CW in morphological, chemical and mechanical traits compared to NW and OW. However, except for an anatomical study in a Korean pine branch (Lee & Eom 1988) and measurement of MFA in a Norway spruce branch (Farber *et al.* 2001), characterization of tracheids and mechanical properties of conifer branch wood remains very limited in the literature.

Radiata pine (*Pinus radiata*) is the most important conifer species for commercial forestry in Australia, New Zealand and Chile. The first two generations of the radiata pine breeding programmes in Australia have improved growth rate by 33% (Wu *et al.* 2007). As a consequence, about half of a harvested log is juvenile wood (Wu *et al.* 2007) that contains up to 44% of CW (Zobel & McElwee 1958). CW and OW have been studied in radiata pine using naturally and artificially bent trunks at different ages and growing conditions (Singh & Donaldson 1999; Donaldson *et al.* 2004; Kibblewhite *et al.* 2005; Tarmian & Azadfallah 2009; Diaz-Vaz *et al.* 2009; Lachenbruch *et al.* 2010). However, reaction wood formed in the bent trunks has assembled effects of gravity stimulus and external bending forces. To gain further insights into CW formed under gravity stimulus, eight tracheid and wood traits were characterized in six radiata pine branches at high resolution using SilviScan® technology (Evans 1994; Evans *et al.* 2000). Our aim is to reveal anatomical and mechanical properties of CW formed in conifer branches with a view to plant gravitropism.

MATERIALS AND METHODS

Plant materials and sampling

A radiata pine commercial plantation aged 13 years (plus two years old seedlings) and located at Bondo, NSW, Australia (35° 16' 44.04" S, 148° 26' 54.66" E) was used for study. Six trees with well-developed branches were selected for the sampling of branch wood. The largest branch was cut from each tree. These branches were generally located at about 1 m above the ground and their angles were approximately 90 degrees. One wood disc (about 5 cm in length) was collected from the cross section of the larger end of each branch. The upper side and underside were immediately labelled on branch discs. Diameters of the discs ranged from 3.5 to 6.2 cm along the upper side to underside direction with annual rings between 9 and 11.

Measurements of tracheid and wood traits

After removing the bark from each disc, a block of wood (about 2 cm in both tangential and longitudinal directions) was cut along the direction from the shortest radius of the upper side to the longest radius of the underside through the pith. A twin-blade saw was used to trim the wood blocks to produce strips (containing pith) 2 mm in the tangential direction and 7 mm in the longitudinal direction. The wood strips were characterised using the SilviScan® instrument (Evans 1994; Evans *et al.* 2000). A total of eight tracheid and wood traits were measured: tracheid wall thickness, radial diameter, tangential diameter, coarseness (tracheid mass per unit length), specific surface area (tracheid surface area per unit mass), cellulose microfibril angle (MFA) (corrected for tracheid orientation), wood density (oven-dried mass per unit volume of air-dried wood) and estimated stiffness (or modulus of elasticity, MOE). All traits except for MFA and MOE were measured at 25 μm intervals in the radial direction. MFA and MOE were measured at 100 μm intervals using a 200 μm diameter X-ray beam and put on the same 25 μm scale as the other traits.

Data analysis

High resolution SilviScan profiles of all eight traits were plotted to show their trends across the wood strips. Underside and upperside wood were compared for each trait in all rings as a whole and in individual rings. The contrast between CW and OW was normalized for each trait using (CW-OW)/OW × 100%. Statistical significances were evaluated using t-tests (paired samples for means) and P values.

RESULTS

Eccentric growth and colour change in branch wood

Eccentric growth was observed in the cross section of the six branch discs (Fig. 1a). The average underside radius was 2.70 cm, significantly longer than the upper side radius (1.77 cm) (P-value ≤ 0.01). Individual underside rings were also significantly wider than the corresponding upper side rings ($P \leq 0.05$) except for the four rings (from pith) and the outermost ring (Fig. 1b). CW in the underside of the six branches (all rings) was dark brown in colour, which was in contrast with the light straw colour of the OW formed in the upper sides (Fig. 1a). In particular, the CW colour was darker in the rings close to the bark than that in the inner rings.

SilviScan profiles of branch wood

SilviScan analysis of the eight tracheid and wood traits for the six branch wood strips were summarised in high resolution profiles. SilviScan profiles of two branch wood strips are shown as examples (Fig. 2). In all traits except for MFA and tracheid tangential diameter the upper side of the branches showed typical ring patterns because

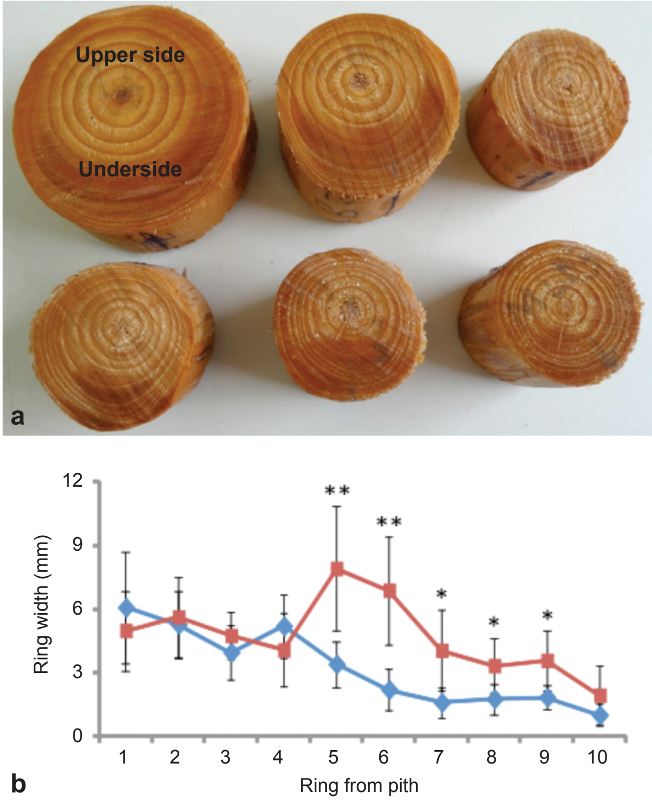

Figure 1. Growth of compression wood (CW) and opposite wood (OW) formed in the six branches. – **a**: Eccentric growth and colour change in branch wood. – **b**: Comparison of growth between CW and OW. Average ring width of the six branches was presented for each ring. Error bars represent one standard deviation. Significant differences at P-values ≤ 0.01 and 0.05 were indicated using ∗∗ and ∗, respectively.

earlywood (EW) and latewood (LW) were clearly separated. These ring patterns were similar to those observed in normal wood (NW) in general. In contrast, seasonal pattern of wood formation in the underside was disrupted by CW formation, causing the wood structure to be more uniform than that in OW, particularly in outer rings from cambial age 4 (Fig. 2).

Comparison of CW and OW formed in branch wood

In terms of average ring values, CW tracheids had significantly thicker walls, higher MFA, lower specific surface area, higher coarseness, higher density and lower stiffness (MOE) compared with OW tracheids (P-value ≤ 0.001) (Table 1). For example, average MFA in the underside of the branches was 35.5 degrees, significantly larger than that in the upper side (28.4 degrees). Differences were also significant between CW and OW in individual rings of age 5–10 (P-value ≤ 0.05) (Fig. 3). In contrast, underside and

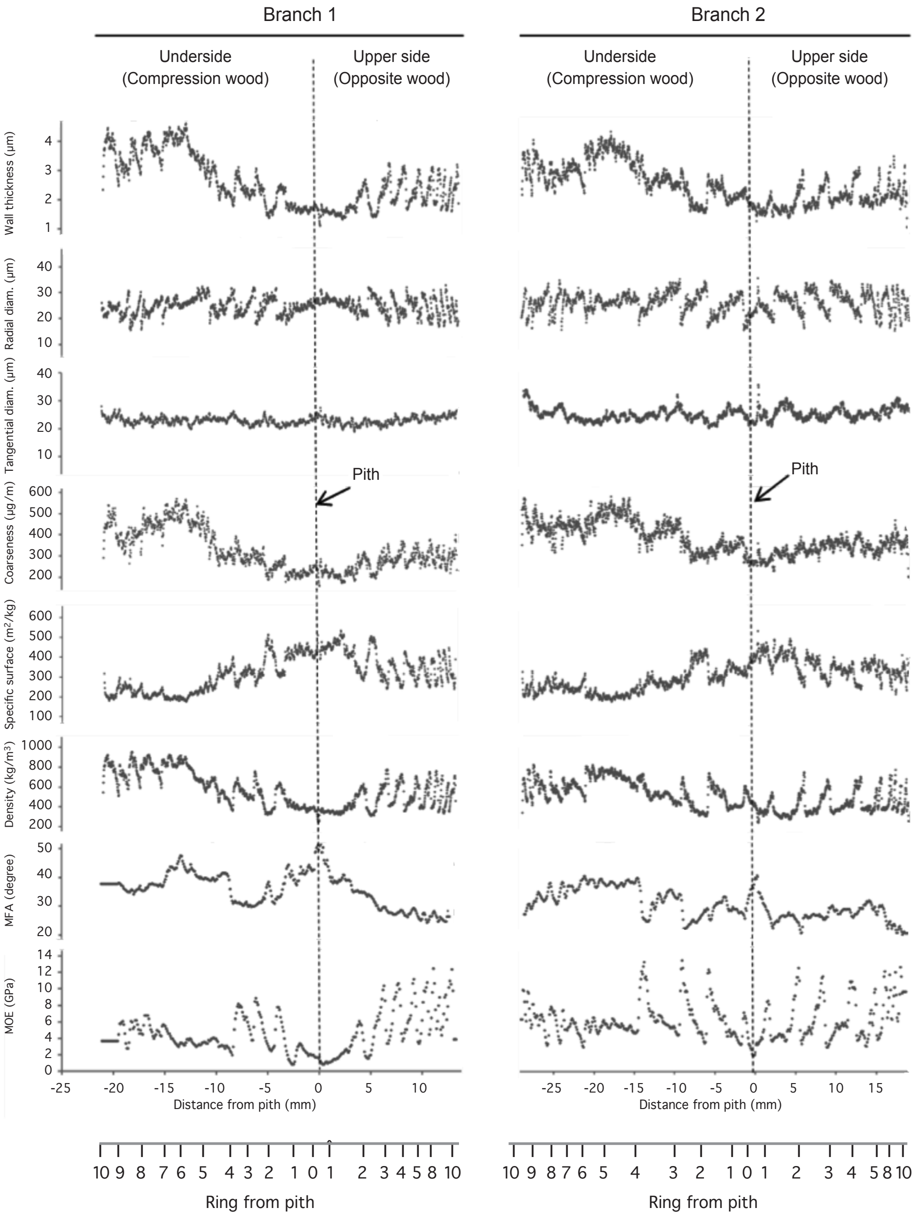

Figure 2. SilviScan profiles of two branch wood strips. Eight tracheid and wood traits were measured in the six branch wood strips at high resolution with 25 μm interval using the SilviScan® technology, including tracheid wall thickness, radial and tangential diameters, coarseness, specific surface, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). SilviScan profiles of two of the six branch wood strips were present in this figure as examples.

upper side differences were generally small for most traits in the innermost four rings (Fig. 3 and Fig. 4), indicating little CW formation in very young branches. Notably, significant increase of MFA was observed in CW except for the first ring from the pith (Fig. 3). Unlike the other six traits, tracheid radial and tangential diameters in the underside wood were not significantly different from those in the upper side wood (Table 1, Fig. 3 and Fig. 4), suggesting that gravitropic stimulus had limited influence on tracheid cell expansion in these samples. In addition, tracheid radial diameter was similar to tangential diameter in both underside and upper side wood in all rings (Table 1 and Fig. 3), resulting in round or square shapes of tracheids formed in branches.

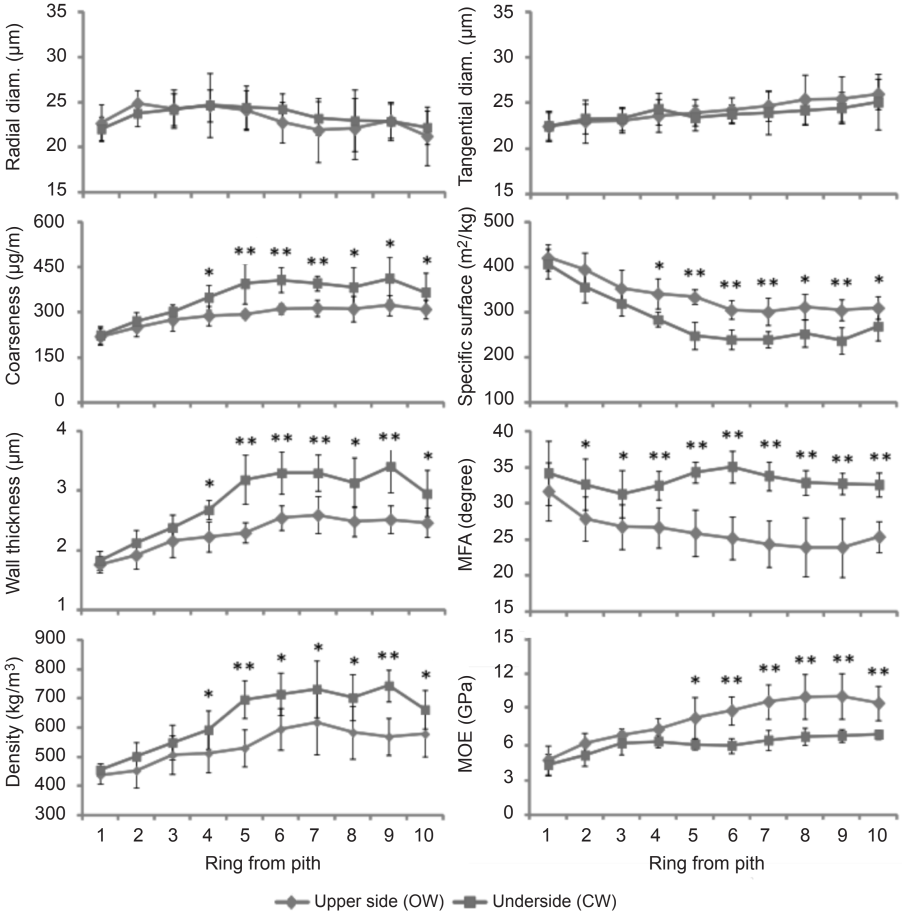

Figure 3. Comparison of compression wood (CW) and opposite wood (OW) formed in six branches in terms of individual rings. Eight tracheid and wood traits were measured by SilviScan®: tracheid wall thickness, radial and tangential diameters, coarseness, specific surface area, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Error bars refer to one standard deviation about the mean values calculated from the six branches. Significant differences at P-values ≤ 0.01 and ≤ 0.05 were indicated using ∗∗ and ∗, respectively.

Table 1. Average ring values of eight tracheid and wood traits in compression wood (CW) and opposite wood (OW) formed in the six branches.

	Wall thickness (μm)	Radial diameter (μm)	Tangential diameter (μm)	Coarseness (μg/m)	Specific surface area (m^2/kg)	MFA (degree)	Density (kg/m^3)	MOE (GPa)
CW	2.9	23.5	24.2	358.9	281.3	35.5	637.8	6.4
OW	2.3	23.1	24.2	292.4	334.8	28.4	542.1	8.5
P-value	< 0.001	0.148	0.961	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

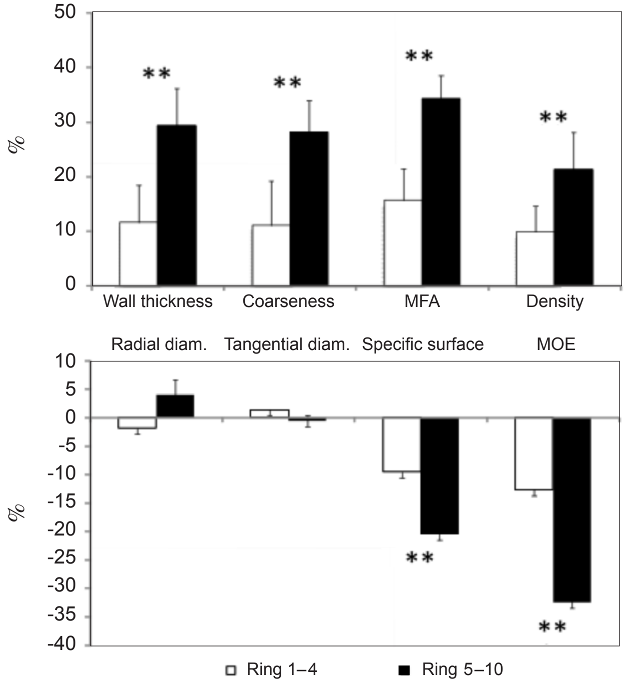

Figure 4. Variation of compression wood (CW) and opposite wood (OW) in the branches. Variation of CW and OW in each of the eight tracheid and wood traits was calculated using (CW-OW)/OW × 100 (%). The traits are tracheid wall thickness, radial and tangential diameters, coarseness, specific surface area, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). CW and OW variation in branches was calculated for younger (1–4 years) and older (5–10 years) ages, respectively. Error bars refer to one standard deviation. Significant differences at P-values ≤ 0.01 and ≤ 0.05 were indicated using ∗∗ and ∗, respectively.

DISCUSSION

Gravity induced eccentric growth in radiata pine branches with increased growth of CW formed on the undersides. This CW formation could generate compression stress to maintain branches at normal orientations. The dark brown colour of CW reflects metabolic pathway changes during reaction wood formation. These include greater lignin biosynthesis (Timell 1986; Lohrasebi *et al.* 1999; Yeh *et al.* 2005; Tarmian & Azadfallah 2009) and thicker secondary cell walls as shown in the present study and elsewhere (Timell 1986; Lee & Eom 1988; Tarmian & Azadfallah 2009; Donaldson & Singh 2013). Higher lignin content in CW absorbs more light, and thicker tracheid walls scatter less light, both of which contribute to the dark brown colour (Tarmian & Azadfallah 2009). Different extractives and metabolites accumulated in CW and OW (Yeh *et al.* 2006b; Diaz-Vaz *et al.* 2009; Tarmian & Azadfallah 2009) may also lead to wood colour changes during reaction wood formation.

Compared to OW tracheids formed in the radiata pine branches, CW tracheids showed thicker walls, higher coarseness, higher density, higher MFA, but lower MOE and specific surface area. Some of these results are in agreement with those from previous studies of bent trunks (Yoshizawa *et al.* 1987; Donaldson *et al.* 2004; Kibblewhite *et al.* 2005; Diaz-Vaz *et al.* 2009; Tarmian & Azadfallah 2009; Lachenbruch *et al.* 2010) and branches (Lee & Eom 1988; Farber *et al.* 2001) of conifer species. The higher density of CW coincided with its thicker tracheid walls as shown in this study, and higher lignin content observed elsewhere (Timell 1986; Donaldson & Singh 2013). Density and MFA are the two major component traits controlling wood stiffness, together accounting for 96% of the variation in stiffness, while MFA alone explained about 86% of the variation (Evans & Ilic 2001). The lower MFA and higher MOE in OW compared to CW plays an important role in tension to hold up branches and bent trunks against gravitational or bending forces (Farber *et al.* 2001), while the greater growth rate in the underside wood (CW) generates compression force that helps to push them upwards. In angiosperms, tension wood (TW) in the upper side of branches has very low MFA and high stiffness compared to OW on the underside (Washusen *et al.* 2005; Qiu *et al.* 2008). Thus, gymnosperms and angiosperms have somewhat comparable similar mechanisms to maintain normal orientation of branches.

In the present study, CW and OW tracheids had similar radial and tangential dimensions, thus, gravitropic stimuli may have a limited impact on primary cell wall expansion. As CW tracheids are shorter than OW tracheids (Timell 1986; Tarmian & Azadfallah 2009), the increased growth of CW in the radiata pine branches (Table 1) could be a result of its rapid cell division at the early development stage. CW formed in the radiata pine branches had much lower stiffness than in the corresponding OW (Table 1 and Fig. 4) and markedly higher MFA even in the younger branches (age 1–4) with little CW formation. These results are in agreement with previous studies of angiosperm branches (Washusen *et al.* 2005; Qiu *et al.* 2008). Therefore, compression wood naturally occurring in branches could be an ideal experimental material for the investigation of cellulose microfibril orientation at the molecular level with a view to plant gravitropism (Qiu *et al.* 2008; Li *et al.* 2013).

CONCLUSIONS

The first high resolution measurements of eight tracheid and wood traits in radiata pine branches revealed that compression wood had relatively increased growth rate, darker brown colour, thicker tracheid walls, higher coarseness, higher microfibril angle, higher wood density, lower stiffness, lower specific surface area, but similar tracheid diameters in both radial or tangential directions. These results indicated that gravity enhanced cell division and secondary cell wall deposition rates in compression wood tracheids but had limited impact on primary wall expansion. Radiata pine branches with marked changes in tracheids and wood traits will facilitate further study on the molecular mechanisms of compression wood formation in conifers.

ACKNOWLEDGEMENTS

This work was part of the Juvenile Wood Initiative (JWI) (PNC050-0304), a project funded by Forest and Wood Products Australia (FWPA), ArborGen LLC, the Southern Tree Breeding Association (STBA), the Queensland Department of Primary Industry (QDPI) and the Commonwealth Scientific and Industrial Research Organization (CSIRO). The authors thank Simon Southerton and Aljoy Abarquez for the assistance in field sampling and wood strip preparation.

REFERENCES

Akbulut T & Ayrilmis N. 2006. Effect of compression wood on surface roughness and surface absorption of medium density fiberboard. Silva Fennica 40: 161–167.

Ban WP, Mancosky D & Lucia LA. 2004. Evaluation of the pulping response of juvenile and mature black spruce compression wood. Cellulose Chem. & Technol. 38: 79–85.

Bendtsen BA. 1978. Properties of wood from improved and intensively managed trees. Forest Prod. J. 28 (10): 61–72.

Brandstrom J. 2004. Microfibril angle of the S_1 cell wall layer of Norway spruce compression wood tracheids. IAWA J. 25: 415–423.

Diaz-Vaz JE, Ananias RA, Rodriguez S, Torres M, Fernandez A & Poblete H. 2009. Compression wood in *Pinus radiata* II: density and chemical composition. Maderas-Ciencia y Tecnologia 11: 139–151.

Donaldson LA, Grace J & Downes GM. 2004. Within-tree variation in anatomical properties of compression wood in radiata pine. IAWA J. 25: 253–271.

Donaldson LA & Singh AR. 2013. Formation and structure of compression wood. In: Fromm J (ed.), Cellular aspect of wood formation (Plant Cell Monographs): 225–256. Springer-Verlag, Berlin.

Evans R. 1994. Rapid measurement of the transverse dimensions of tracheids in radial wood sections from *Pinus radiata*. Holzforschung 48: 168–172.

Evans R & Ilic J. 2001. Rapid prediction of wood stiffness from microfibril, angle and density. Forest Prod. J. 51: 53–57.

Evans R, Stringer S & Kibblewhite RP. 2000. Variation of microfibril angle, density and fibre orientation in twenty-nine *Eucalyptus nitens* trees. Appita J. 53: 450–457.

Farber J, Lichtenegger HC, Reiterer A, Stanzl-Tschegg S & Fratzl P. 2001. Cellulose microfibril angles in a spruce branch and mechanical implications. J. Materials Sci. 36 (21): 5087–5092.

Gindl W. 2002. Comparing mechanical properties of normal and compression wood in Norway spruce: The role of lignin in compression parallel to the grain. Holzforschung 56: 395–401.

Kibblewhite RP, Evans R, Grace JC & Riddell MJC. 2005. Fibre length, microfibril angle and wood colour variation and interrelationships for two radiata pine trees with mild and severe compression wood. Appita J. 58: 316–322.

Lachenbruch B, Droppelmann F, Balocchi C, Peredo M & Perez E. 2010. Stem form and compression wood formation in young *Pinus radiata* trees. Canad. J. Forest Res. – Revue Canadienne de Recherche Forestière 40: 26–36.

Lee PW & Eom YG. 1988. Anatomical comparison between compression wood and opposite wood in a branch of Korean pine (*Pinus koraiensis*). IAWA Bull. n.s. 9: 275–284.

Li XG, Yang XH & Wu HX. 2013. Transcriptome profiling of radiata pine branches reveals new insights into reaction wood formation with implications in plant gravitropism. BMC Genomics 14: 768.

Lohrasebi H, Mabee WE & Roy DN. 1999. Chemistry and pulping feasibility of compression wood in black spruce. J. Wood Chem. & Technol. 19: 13–25.

Nicholls JWP. 1982. Wind action, leaning trees and compression wood in *Pinus radiata* D. Don. Austral. Forest Res. 12: 75–92.

Qiu D, Wilson IW, Gan S, Washusen R, Moran GF & Southerton SG. 2008. Gene expression in *Eucalyptus* branch wood with marked variation in cellulose microfibril orientation and lacking G-layers. New Phytol. 179: 94–103.

Singh AP & Donaldson LA. 1999. Ultrastructure of tracheid cell walls in radiata pine (*Pinus radiata*) mild compression wood. Canad. J. Bot. – Revue Canadienne de Botanique 77: 32–40.

Tarmian A & Azadfallah M. 2009. Variation of cell features and chemical composition in spruce consisting of opposite, normal, and compression wood. Bioresources 4: 194–204.

Timell TE. 1986. Compression wood in gymnosperms. Springer-Verlag, Berlin.

Warensjo M & Rune W. 2004. Stem straightness and compression wood in a 22-year-old stand of container-grown Scots pine trees. Silva Fennica 38: 143–153.

Washusen R, Evans R & Southerton S. 2005. A study of *Eucalyptus grandis* and *Eucalyptus globulus* branch wood microstructure. IAWA J. 26: 203–210.

Wu HX, Eldridge KG, Matheson AC, Powell MB, McRae TA, Butcher TB & Johnson IG. 2007. Achievements in forest tree improvement in Australia and New Zealand: 8. Successful introduction and breeding of radiata pine in Australia. Austral. Forestry 70: 215–225.

Yamashita S, Yoshida M, Takayama S & Okuyama T. 2007. Stem-righting mechanism in gymnosperm trees deduced from limitations in compression wood development. Ann. Bot. 99: 487–493.

Yamashita S, Yoshida M & Yamamoto H. 2009. Relationship between development of compression wood and gene expression. Plant Science 176: 729–735.

Yeh TF, Braun JL, Goldfarb B, Chang HM & Kadla JF. 2006a. Morphological and chemical variations between juvenile wood, mature wood, and compression wood of loblolly pine (*Pinus taeda* L.). Holzforschung 60: 1–8.

Yeh TF, Goldfarb B, Chang HM, Peszlen I, Braun JL & Kadla JF. 2005. Comparison of morphological and chemical properties between juvenile wood and compression wood of loblolly pine. Holzforschung 59: 669–674.

Yeh TF, Morris CR, Goldfarb B, Chang HM & Kadla JF. 2006b. Utilization of polar metabolite profiling in the comparison of juvenile wood and compression wood in loblolly pine (*Pinus taeda*). Tree Physiol. 26: 1497–1503.

Yoshizawa N, Kiyomiya M & Idei T. 1987. Variations in tracheid length and morphological changes in tracheid tips associated with the development of compression wood. Wood Sci. & Technol. 21: 1–10.

Zobel BJ & McElwee RL. 1958. Variation of cellulose in loblolly pine. Tappi J. 41: 167–170.

Accepted: 4 April 2014