3rd New Zealand and German CELL WALL SYMPOSIUM

SCION Next generation biomaterials

New Zealand Journal of Forestry Science

39 (2009) 161-168

http://nzjfs.scionresearch.com

Immunocytochemistry of Xylem Cell Walls†

Lloyd A. Donaldson

Scion, Private Bag 3020, Rotorua 3046, New Zealand
Lloyd.donaldson@scionresearch.com

(Submitted for publication 8 December 2008; accepted in revised form 18 August 2009)

Abstract

Immunocytochemistry uses labelled antibodies to localise specific cell wall components, either by fluorescence microscopy or by electron microscopy. Antibodies have been developed to detect both polysaccharides and lignin moieties, allowing visualisation of cell wall components with high resolution and specificity. Similar techniques have been developed to use enzymes or carbohydrate binding modules with specific affinity to cell wall components. This review summarises progress in this field as applied to xylem cell walls, outlining current knowledge and exploring the scope for future investigations.

Keywords: Cell walls, xylem; immunocytochemistry; antibody; carbohydrate binding module; fluorescence microscopy; electron microscopy.

† Based on a paper presented at the 3rd Joint New Zealand-German Symposium on Plant Cell Walls, 13-15 February 2008, Auckland, New Zealand

Introduction

The specific binding properties of animal antibodies have long been used to localise proteins in animal and plant cells when combined with fluorescent or electron dense labels. The use of antibodies to localise polysaccharides and polyphenolic cell wall components is a more specialised application which has only recently started to be applied to the understanding of wood cell walls (Kim & Koh, 1997; Ruel et al., 2002; Kukkola et al., 2003; Joseleau et al., 2004a; Hafren, 2005; Altaner, Hapca et al., 2007; Altaner, Knox et al., 2007; Bowling & Vaughn, 2008). The use of defined monoclonal antibodies with a high level of specificity enables very precise localisation of cell wall components that cannot readily be achieved by other approaches such as spectroscopy or the use of specific reagents. The precision of immunolocalisation makes it an essential tool for functional analysis, understanding cell wall assembly, and for phenotypic analysis in genetic modification experiments (Willats et al., 2000). Much of this work has focussed on primary cell walls but has increasingly been applied to secondary cell walls, wood and wood fibres in tree species.

Although extremely useful, the use of antibodies to detect and locate cell wall components has been limited by various technical problems in raising antibodies to polysaccharides and phenolics.

- Polysaccharides have inherently low immunogenicity compared with proteins. Also, it is more difficult to raise antibodies against relatively small molecules compared to proteins and this can often require linking specific oligosaccharides to a protein carrier molecule known as a neoglycoprotein. When successful, this may lead to clear, unambiguous localisation of a polysaccharide in the cell wall.

- The lack of a robust fixation method for cell wall components may mean that soluble components

ISSN 0048 - 0134

are lost to at least some degree. In this case, tissue printing may be a useful procedure, but this is probably less of a problem in lignified secondary cell walls, where potentially soluble components are likely to be bound by the lignin. Masking of polysaccharide epitopes by lignin is a potential problem when dealing with wood cell walls.

As alternatives to antibodies, researchers have begun to exploit the fact that many microorganisms have evolved enzymes that degrade plant cell wall components. Such enzymes may have non-catalytic components that facilitate enzyme binding to the substrate. These structures are known as carbohydrate binding modules (CBMs). Carbohydrate binding modules have been identified with specificity for cellulose, xylans, mannans, glucomannans and β-(1→3)-glucans (Boraston et al., 2004; Knox & McCartney 2005). Recombinant histidine-tagged CBM fusion proteins can be applied in a similar way to antibodies in localisation experiments. Carbohydrate binding modules can often recognise subtle differences among similar polymers, e.g. crystalline versus amorphous cellulose (McCartney et al., 2004; Knox & McCartney, 2005). Carbohydrate binding modules have recently been applied to wood and fibre studies (Daniel et al., 2006; Filonova et al., 2007).

Polysaccharides

Pectins

Pectic polysaccharides, some of the most complex macromolecules in nature, occur in the primary walls of developing xylem cells (Vincken et al., 2003). Pectins have a role in cell adhesion, cell elongation, maintenance of structure and stability, and in mature xylem tissue, the pectin gel becomes infiltrated with large amounts of lignin (Donaldson, 2001).

Homogalacturonans are found in nearly all primary walls. Homogalacturonans have been immunolocalised in the primary walls of xylem in conifers including Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Japanese cedar (*Cryptomeria japonica* (L.f.) D.Don), and have been studied in radiata pine (*Pinus radiata* D.Don) cambium using antibodies with specificity to the methyl-esterified (JIM7) and unesterified (JIM5) components (Putoczki et al., 2008). Pectins are localised to the compound middle lamella of developing tracheids and ray cells, as well as pit membranes. Labelling declines with increasing maturity of tracheids, possibly due to masking of the epitope by lignin (Maeda et al., 2000). Antibodies to methyl-esterified and unesterified components show a similar distribution but differ in intensity. Similar results are found in Scots pine (*Pinus sylvestris* L.) developing xylem (Hafrén et al., 2000). In lignified xylem, the methyl-esterified form of pectin seems to dominate. Using an antibody to acidic galacturonan (PAM1) and to partially methyl-esterified galacturonan (JIM7), Hafren (2005) demonstrated the de-esterification of galacturonan on pulp fibre surfaces as a result of alkaline hydrogen peroxide bleaching, using an alkaline phosphatase assay.

Using an indirect approach, Daniel et al. (1996) were able to study the sites of pectinase activity on pit membranes in Scots pine sapwood using antibodies to the enzyme combined with colloidal gold immunolocalisation. Both pectinase (and cellulase) appeared to penetrate into the pit membrane during enzymatic hydrolysis. In poplar (*Populus* spp.), Guglielmino et al. (1997) also found unesterified pectins in the middle lamella at cell junctions of differentiating tracheids, but methyl-esterified pectins increased relative to unesterified pectins as cell walls became lignified. JIM5 antibody binding was lost from radial and tangential primary walls in lignified cell walls suggesting a loss of unesterified pectins from these regions, but remained at reduced levels in the cell junctions. RG-I-Type pectin molecules have also been localised in the gelatinous layer of tension wood fibres in Sweet-gum (*Liquidambar styraciflua* L.) and Hackberry (*Celtis occidentalis* L.) (Bowling & Vaughn, 2008).

Mannans

Glucomannans are the dominant hemicellulose in softwood tracheids. Prior to the development of antibody techniques, Ruel and Joseleau (1984) used mannanase-gold complexes to localise glucomannans in spruce (*Picea* spp.) tracheids. Mannan was localised across the cell wall of the tracheids but mainly in the secondary wall. Hosoo et al. (2002; 2006a,b) studied the distribution of glucomannans in developing tracheids of Japanese cedar and found a diurnal pattern of hemicellulose deposition during the dark period, and cellulose deposition in the light period, that may be associated with changes in turgor pressure. Glucomannans were present in the secondary wall in conjunction with xylans. Maeda et al. (2000), using a polyclonal antibody to glucomannans, found that labelling was restricted to the secondary wall of developing tracheids in Hinoki cypress (*Chamaecyparis obtusa* (Siebold & Zucc.) Endl). The amount of labelling was found to decline as tracheids became lignified. Altaner, Knox, et al., (2007) studied mannan distribution in Sitka spruce (*P. sitchensis* (Bong.) Carrière) using immunofluorescence microscopy and found labelling of secondary walls in both normal and compression wood tracheids but with reduced labelling of resin canals.

Mannans are less abundant in hardwood xylem, but Handford et al. (2003) used antibodies with affinity against β-(1→4)-mannosyl residues to localise mannans in the secondary walls of xylem, xylem parenchyma and interfascicular fibres of *Arabidopsis* (*A. thaliana* (L.) Heynh.) inflorescence stems by using immunofluorescence and immunogold techniques.

 ISSN 0048 - 0134

Xylans

Hardwood fibres contain hemicellulose rich in glucuronoxylans (Figure 1). Studies with antibodies to unsubstituted or low substituted xylans (LM10), and arabinoxylan (LM11) have shown the presence of both epitopes in secondary cell walls of xylem in flax and tobacco (McCartney et al., 2005). In addition to secondary walls of tracheids, LM11 shows strong binding to resin canals in Sitka spruce (Altaner, Knox, et al., 2007). The presence of xyloglucan in the G-layer of tension wood fibres has been inferred by the immunodetection of xyloglucan endo-transglycosylase and xyloglucan endo-transglycosylase/hydrolase in poplar tension wood (Nishikubo et al., 2007). Using antibodies to both mannan and xylan, Hosoo et al. (2006a,b) have shown a diurnal pattern in hemicellulose deposition in Japanese cedar.

Glucuronoxylans have been immunolocalised in the secondary cell walls of Japanese beech (*Fagus crenata* Blume) fibres (Awano et al., 1998). Glucuronoxylans were uniformly distributed across the secondary cell wall but were less abundant in the outer layer. Labelling density was found to increase during maturation of developing fibres and the authors concluded that deposition occurs by infiltration of the wall in addition to deposition at the lumen surface as the wall is formed. In contrast, Ruel et al., (2006) found that two structurally different xylans were deposited at different times in conjunction with lignin deposition in *Arabidopsis* and poplar. In poplar, unsubstituted xylans are deposited predominantly in the S1 and S3 layers (Ruel et al., 2006). Xylan was found to be abundant in the secondary thickening of *Zinnia* (*Z. elegans* L.) tracheary element cultures (Northcote et al., 1989). Filonova et al. (2007) also demonstrated binding of labelled xylan binding modules to birch (*Betula spp*.), pine and poplar wood sections, and to various pulp fibre types. In this study, a range of synthetic binding modules was used, showing different staining patterns on wood sections, suggesting that different modules have different affinities. Treatments such as delignification resulted in much higher levels of binding.

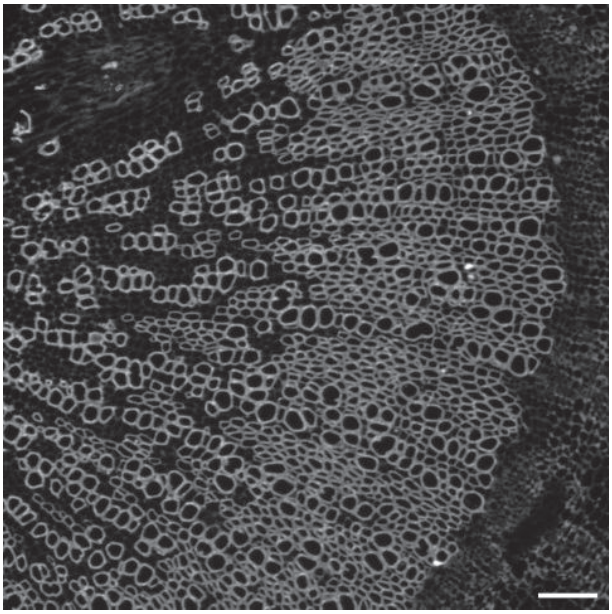

FIGURE 1: Distribution of xylan in *Arabidopsis* secondary xylem using LM10 localised with an Alexa 568 labelled secondary antibody using confocal fluorescence microscopy. Scale bar = 49 µm.

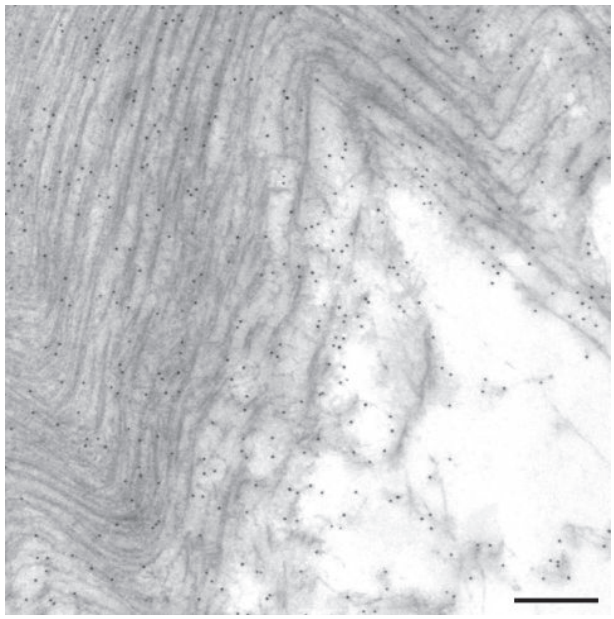

FIGURE 2: Immunolocalisation of galactan (LM5) with a 15 nm gold-labelled secondary antibody in the unlignified secondary wall of developing radiata pine compression wood. Scale bar = 375 nm.

Galactan

β-(1→4)-Galactan occurs in the secondary walls of compression wood tracheids (Figure 2). Schmitt et al. (2006) demonstrated the localisation of galactan in the outer S2 layer of radiata pine compression wood using LM5 antibody. Altaner, Hapca, et al., (2007) have also localised galactan using the LM5 antibody labelled with FITC (fluoroscein isothiocyanate). Galactan was found only in the outer secondary wall (S2L) of fully developed compression wood. However, results from our laboratory have shown that in unlignified developing compression wood xylem, galactan is distributed throughout the secondary wall, and that lignification masks galactan localisation using the LM5 antibody (Mast et al., 2009). This result was confirmed by both fluorescence and electron microscopy. Arend (2008) studied the distribution of β-(1→4)-galactan in tension wood fibres of poplar and found a highly localised distribution in the boundary layer between the gelatinous layer and the secondary cell wall, suggesting a role in cross-linking between the two layers.

 ISSN 0048 - 0134

Cellulose

Some early attempts at localising cellulose used antibodies to specific cellulase enzymes coupled with immunofluorescence (Taylor et al., 1992; Daniel et al., 1996). Taylor et al. (1992) showed that deposition of cellulose and xylan is required to achieve normal localised lignin deposition in *Zinnia* tracheary elements. Later studies using the *Arabidopsis* mutant *irx3* showed that fibre walls can form in secondary xylem in the absence of cellulose although the ultrastructure is abnormal (Taylor et al., 2000).

While no one has successfully raised antibodies to crystalline cellulose, the recent development of cellulose binding modules cloned from cellulolytic enzymes has allowed specific detection of cellulose using both fluorescence and electron microscopy (Boraston et al., 2004; McCartney et al., 2004; Knox & McCartney, 2005; Daniel et al., 2006). In tension wood of poplar and birch, crystalline cellulose was localised to the G-layer. Reduced labelling was also found on the lignified secondary wall of these fibres. Using a range of cellulose binding domains with differential affinity for crystalline and non-crystalline cellulose, Altaner, Knox, et al. (2007) found varying levels of binding to Sitka spruce tracheids.

Pinto et al., (2008) have used fluorescently labelled cellulose binding domains to study the distribution of binding domains on the surface of cellulose fibres. It was demonstrated that the binding domains, in fact, are able to penetrate the fibres in relatively large quantities.

Other Polysaccharides

Arabinan and callose have both been localised in wood using immuno-techniques. Altaner, Knox, et al. (2007) localised α-(1→5)-arabinan (LM6) at the cell junctions of compression wood tracheids in Sitka spruce lining the intercellular spaces, while β-(1→3)-glucan (callose) was localised to the helical cavities in the secondary wall of the same sample.

Lignin

Immunolocalisation of lignin has focussed on different chemical epitopes within the polymer. Ruel et al. (2002) have investigated the distribution of condensed (containing difficult to cleave linkages) and non-condensed (containing easy to cleave linkages) units of mixed guaiacyl/syringyl lignins in xylem from tobacco (*Nicotiana tabacum* L.) and *Arabidopsis* plants genetically modified for down-regulated cinnamoyl CoA reductase (CCR). These plants showed a reduction in non-condensed lignin and associated delamination of secondary cell walls. The presence of significant amounts of condensed lignin epitope in the delaminating regions suggests that it is the non-condensed lignin that provides the normal cohesion of cellulose microfibrils.

Goujon et al. (2003) also studied *Arabidopsis* xylem in plants down-regulated for cinnamoyl CoA reductase, the first enzyme in the lignin biosynthetic pathway. These plants showed a 50% reduction in lignification, and delamination of vessel and interfascicular fibre cell walls as well as incorporation of ferulic acid into the cell walls. Immunocytochemistry using immunogold electron microscopy with antibodies against non-condensed guaiacyl/syringyl lignin sub units, showed a reduction in the epitope in interfascicular fibres and xylem in the transformed plants. Non-condensed lignin epitopes were concentrated in the S1 region of fibres compared to a uniform labelling of the secondary wall in wild type controls. Labelling was absent in vessels in the transformed plants.

Condensed (guaiacyl and guaiacyl/syringyl) and non-condensed (guaiacyl/syringyl) lignins have also been localised in transgenic aspen trees transformed with 35S-*rol*C and exhibiting reduced cambial activity (Grünwald et al., 2001, 2002, 2003). In developing fibres, condensed lignin was deposited in the cell corner middle lamella region while non-condensed lignin was deposited in the secondary wall near the cell corners at the stage of S1 formation. Non-condensed guaiacyl/syringyl lignins were not labelled in the control trees but the transgenic cell walls were densely labelled.

In *Arabidopsis*, Ruel et al. (2006) found evidence for two distinct phases of lignin deposition during fibre wall development with condensed lignins being deposited during the early stages of secondary wall formation, followed by later deposition of non-condensed lignins. This further supports the earlier conclusion (Ruel et al., 2002) that non-condensed lignins are responsible for cell wall cohesion.

Joseleau et al. (2004a) have used a polyclonal antibody against syringylpropane epitopes to localise syringyl lignin in secondary walls of *Arabidopsis*, poplar and tobacco xylem. In *Arabidopsis*, labelling was greater in fibres than vessels, with an increase in labelling across the developing secondary wall towards the lumen and very little labelling in the S1 region. Similar results were found for tobacco, but with the greatest density of label in the outer S2 region of fibres. Poplar was similar to tobacco, but with a more uniform distribution across the S2 region.

Immuno-techniques have been used to demonstrate the presence of mainly non-condensed syringyl epitopes in the inner part of the G-layer of poplar (Joseleau et al., 2004b). In normal wood these syringyl epitopes also accumulate towards the inner part of the S2 region but occur at only low levels in the S2 region of gelatinous fibres. Condensed guaiacyl epitopes were more abundant in the S1 and S2 layers of fibres, with a more or less even distribution in both normal and tension wood, while non-condensed mixed guaiacyl-syringyl epitopes were about twice

 ISSN 0048 - 0134

as common in the S2 as in the S1 or G-layer. The amount of syringyl epitope in gelatinous fibres was reported to be 40% of that in normal wood fibres.

Kukkola et al. (2003) have studied the distribution of dibenzodioxocin dimers in lignifying Norway spruce (*P. abies* (L.) H.Karst.) using transmission electron microscopy and immunogold localisation with a polyclonal antibody raised directly against the dibenzodioxocin dimer conjugated with a lectin. Dibenzodioxocin dimers were localised mainly in the S2 and S3 layers during secondary wall formation, but were also present in the middle lamella. Further studies using fluorescence microscopy showed labelling in the S3 layer of both Norway spruce and silver birch (*B. pendula* Roth). In the case of birch, most of the labelling was in the vessel cell wall (Kukkola et al., 2004). In Norway spruce and Scots pine, dibenzodioxocin dimers show variable distribution/occurrence between juvenile and normal wood and in compression wood (Kukkola et al., 2008). Dibenzodioxocin may be evenly distributed in the S2 layer of juvenile Scots pine tracheids but is only present at low levels in Norway spruce juvenile wood. In compression wood, dibenzodioxocin is present in the S1 layer and outer S2 layer while in Norway spruce the epitope is localised in the inner secondary wall of compression wood tracheids.

In contrast to the above localisations of specific sub-groups, Kim and Koh (1997) used immuno-gold localisation to determine the origin of milled wood lignin in Norway spruce. The antibody was relatively non-specific, showing cross-reactivity with radiata pine, Japanese cedar and birch milled wood lignins. In Norway spruce, the most intense labelling was observed in the S2 region with no labelling in the lignin rich middle lamella region.

Glycoproteins

Bao et al. (1992) demonstrated the presence of extensin-like protein in loblolly pine (*Pinus taeda* L.) tracheids using antibodies raised against purified protein. Using wheat-germ agglutinin, antibodies to wheat germ agglutinin, and antibodies raised against isolated protein, labelled with gold, Wojtaszek and Bolwell (1995) were able to demonstrate the presence of glycoproteins in the secondary cell walls of xylem from French bean (*Phaseolus vulgaris* var. *vulgaris* L.) hypocotyls. A number of studies have localised cell wall structural proteins [extensins, glycine rich proteins (GRP), hydroxyproline-rich glycoproteins (HPRGP), arabinogalactan proteins (AGP)] in primary and secondary xylem of annual plants (Ye & Varner, 1991; Condit et al., 1990; Ye et al., 1991; Keller et al., 1989; Keller & Baumgartner, 1991; Wyatt et al., 1992). Many of these studies have been carried out using tissue printing techniques. A range of cell wall proteins down-regulated by drought were localised to pit membranes and primary walls of tomato xylem using gold immunolocalisation (Harrak et al., 1999).

More recently there has been greater interest in the cell wall proteins that occur in secondary xylem of tree species. Zhang et al. (2003) purified and immunolocalised an arabinogalactan protein in loblolly pine. The epitope was localised specifically to differentiating tracheids in the early stages of secondary wall formation and prior to lignification. Antigenicity was either lost or blocked by lignification in tracheids at more advanced stages of development and in mature xylem. In poplar tension wood, Lafarguette et al. (2004) demonstrated the presence of fasciculin-like arabinogalactan proteins specifically expressed in tension wood but not in the cambial zone. Immunolocalisation (JIM14) revealed that AGP was localised in the inner part of the G-layer of tension wood fibres, and in the middle lamella/primary wall of fibres, vessels and ray parenchyma in opposite wood.

Conclusions

Immunolocalisation of polysaccharide, polyphenolic, and protein cell wall components has provided useful information on the development and topochemistry of secondary cell walls in xylem tissues from a range of plants. Immunolocalisation offers a much greater specificity than other conventional staining methods. There remain many opportunities to study the composition and development of secondary cell walls in the xylem of tree species by the use of fluorescence and electron microscopy using immunolocalisation techniques, including those based on carbohydrate-binding modules. In particular, there are opportunities to develop antibodies to the *p*-hydroxy-rich lignin of compression wood. Immunocytochemistry is a useful method for understanding plant cell walls, their transformation, the associations between polymers within cell walls, and the mechanisms that control localisation of different cell wall components during wood formation.

References

Altaner, C., Hapca, A. I., Knox, J. P., & Jarvis, M. C. (2007). Detection of β-1-4-galactan in compression wood of sitka spruce (*Picea sitchensis* (Bong.) Carriere) by immunofluorescence. *Holzforschung, 61*, 311-316.

Altaner, C., Knox, J. P., & Jarvis, M. C. (2007). *In situ* detection of cell wall polysaccharides in Sitka spruce (*Picea sitchensis* (Bong.) Carrière) wood tissue. *BioResources, 2*, 284-295.

 ISSN 0048 - 0134

Arend, M. (2008). Immunolocalisation of (1,4)-β-galactan in tension wood fibers of poplar. *Tree Physiology, 28*, 1263-1267.

Awano, T., Takabe, K., & Fujita, M. (1998). Localisation of glucuronoxylans in Japanese beech visualised by immunogold labelling. *Protoplasma, 202*, 213-222.

Bao, W., O'Malley, D. M., & Sederoff, R. R. (1992). Wood contains a cell-wall structural protein. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 6604-6608.

Boraston, A. B., Bolam, D. N., Gilbert, H. J., & Davies, G. J. (2004). Carbohydrate-binding modules: Fine-tuning polysaccharide recognition. *Biochemistry Journal, 382*, 769-781.

Bowling, A. J., & Vaughn, K. C. (2008). Immunocytochemical characterisation of tension wood: Gelatinous fibers contain more than just cellulose. *American Journal of Botany, 95*, 655-663.

Condit, C. M., McClean, B. G., & Meagher, R. B. (1990). Characterization of the expression of the petunia glycine-rich protein-1 gene product. *Plant Physiology, 93*, 596-602.

Daniel, G., Filonova, L., Kallas, Å. M., & Teeri, T. T. (2006). Morphological and chemical characterisation of the G-layer in tension wood fibres of *Populus tremula* and *Betula verrucosa*: Labelling with cellulose-binding module CBM1HjCel7A and fluorescence and FE-SEM microscopy. *Holzforschung, 60*, 618–624.

Daniel, G., Singh, A., & Nilsson, T. (1996). Ultrastructural and immunocytochemical studies on the window and bordered pit membranes of *Pinus sylvestris* L. In L. A. Donaldson, A. P. Singh, B. G. Butterfield, & L. J. Whitehouse (Eds.), *Recent advances in wood anatomy* (pp. 373-383). Rotorua, New Zealand New Zealand: Forest Research Institute Limited.

Donaldson, L. A. (2001). Lignification and lignin topochemistry – an ultrastructural view. *Phytochemistry, 57*, 859-873.

Filonova, L., Gunnarsson, L. C., Daniel, G., & Ohlin, M. (2007). Synthetic xylan-binding modules for mapping of pulp fibres and wood sections. *BMC Plant Biology, 7*, 1-10.

Goujon, T., Ferret, V., Mila, I., Pollet, B., Ruel, K., Burlat, V., Joseleau, J. P., Barriere, Y., Lapierre, C., & Jouanin, L. (2003). Down-regulation of AtCCR1 gene in *Arabidopsis thaliana*: effects on phenotype, lignins and cell wall degradability. *Planta, 217*, 218-228.

Grünwald, C., Ruel, K., Joseleau, J.-P., & Fladung, M. (2001). Morphology, wood structure and cell wall composition of *rolC* transgenic and non-transformed aspen trees. *Trees, 15*, 503-517.

Grünwald, C., Ruel, K., & Schmitt, U. (2002). Differentiation of xylem cells in *rolC* transgenic aspen trees – a study of secondary cell wall development. *Annals of Forestry Science, 59*, 679-685.

Grünwald, C., Ruel, K., & Schmitt, U. (2003). Differentiation of xylem cells in *rolC* transgenic Aspen trees. *Annals of Forestry Science, 2*, 14-21.

Guglielmino, N., Liberman, M., Jauneau, A., Vian, B., Catesson, A. M., & Goldberg, R. (1997). Pectin immunolocalisation and calcium visualisation in differentiating derivatives from poplar cambium. *Protoplasma, 199*, 151-160.

Hafren, J. (2005). Antibody-based assay for galacturonan deesterification on wood pulp fibers during bleaching. *Journal of Wood Science, 51*, 655-658.

Hafren, J., Daniel, G., & Westermark, U. (2000). The distribution of acidic and esterified pectin in cambium, developing xylem and mature xylem of *Pinus sylvestris*. *IAWA Journal, 21*, 157-168.

Handford, M. G., Baldwin, T. C., Goubert, F., Prime, T. A., Miles, J., Yu, X., & Dupree, P. (2003). Localisation and characterisation of cell wall mannan polysaccharides in *Arabidopsis thaliana*. *Planta, 218*, 27-36.

Harrak, H., Chamberland, H., Plante, M., Bellemare, G., Lafontaine, J. G., & Tabaeizadeh, Z. (1999). A proline-, threonine- and glycine-rich protein down-regulated by drought is localised in the cell wall of xylem elements. *Plant Physiology, 121*, 557-564.

Hosoo, Y., Imai, T., & Yoshida, M. (2006a). Diurnal differences in the supply of glucomannans and xylans to innermost surface of cell walls at various developmental stages from cambium to mature xylem in *Cryptomeria japonica. Protoplasma, 229*, 11-19.

Hosoo, Y., Imai, T., & Yoshida, M. (2006b). The supply of matrix containing glucomannans to the innermost surface of S2 layers is associated with changes in turgor pressure of differentiating tracheids in *Cryptomeria japonica*. *Journal of Wood Science, 52*, 471-476.

 ISSN 0048 - 0134

Hosoo, Y., Yoshida, M., Imai, T., & Okuyama, T. (2002). Diurnal difference in the amount of immunogold-labelled glucomannans detected with field emission scanning electron microscopy at the innermost surface of developing secondary walls of differentiating conifer tracheids. *Planta, 215*, 1006-1012.

Joseleau, J. P., Faix, O., Kuroda, K.-I., & Ruel, K. (2004a). A polyclonal antibody directed against syringylpropane epitopes of native lignins. *Comptes Rendus Biologies, 327(9)*, 809-815.

Joseleau, J. P., Imai, T., Kuroda, K.-I., & Ruel, K. (2004b). Detection in situ and characterization of lignin in the G-layer of tension wood fibres of *Populus deltoïdes*. *Planta, 219*, 338-345.

Keller, B., & Baumgartner, C. (1991). Vascular-specific expression of the bean GRP 1.8 gene is negatively regulated. *Plant Cell, 3*, 1051-1061.

Keller, B., Templeton, M. D., & Lamb, C. J. (1989). Specific localization of a plant cell wall glycine-rich protein in protoxylem cells of the vascular system. *Proceedings of the National Academy of Sciences of the United States of America, 86*, 1529-1533.

Kim, Y.-S., & Koh, H.-B. (1997). Immuno electron microscopic study on the origin of milled wood lignin. *Holzforschung, 51*, 411-413.

Knox, J. P., & McCartney, L. (2005). Carbohydrate-binding modules as molecular probes for plant cell wall polymers. *Microscopy and Microanalysis, 11*, 1128-1129.

Kukkola, E. M., Koutaniemi, S., Gustafsson, M., Karhunen, P., Ruel, K., Lundell, T., Saranpää, P., Brunow, G., Teeri, T. H., & Fagerstedt, K. V. (2003). Localization of dibenzodioxocin substructures in lignifying Norway spruce xylem by transmission electron microscopy-immunogold labelling. *Planta, 217*, 229-237.

Kukkola, E. M., Koutaniemi, S., Pöllänen, E., Gustafsson, M., Karhunen, P., Lundell, T. K., Saranpää, P., Kilpelainen, I., Teeri, T. H., & Fagerstedt, K. V. (2004). The dibenzodioxocin lignin substructure is abundant in the inner part of the secondary wall in Norway spruce and silver birch xylem. *Planta, 218*, 497-500.

Kukkola, E., Saranpää, P., & Fagerstedt, K. (2008). Juvenile and compression wood cell wall layers differ in lignin structure in Norway spruce and Scots pine. *IAWA Journal, 29*, 47-54.

Lafarguette, F., Leplé J.-C., Déjardin, A., Laurans, F., Costa, G., Lesage-Descauses, M.-C., & Pilate, G. (2004). Poplar genes encoding fasciculin-like arabinogalactan proteins are highly expressed in tension wood. *New Phytologist, 164*, 107-121.

Maeda, Y., Awano, T., Takabe, K., & Fujita, M. (2000). Immunolocalisation of fucomannans in the cell wall of differentiating tracheids in *Chamaecyparis obtusa*. *Protoplasma, 213*, 148-156.

Mast, S. W., Donaldson, L. A., Torr, K., Phillips, L., Flint, H., West, M., Strabala, T. J., & Wagner, A. (2009). Exploring the ultrastructural localisation and biosynthesis of β(1,4)-Galactan in *Pinus radiata* compression wood. *Plant Physiology, 150*, 573-583.

McCartney, L., Gilbert, H. J., Bolam, D. N., Boraston, A. B., & Knox, J.P. (2004). Glycoside hydrolase carbohydrate-binding modules as molecular probes for the analysis of plant cell wall polymers. *Analytical Biochemistry, 326*, 49-54.

McCartney, L., Marcus, S. E., & Knox, J. P. (2005). Monoclonal antibodies to plant cell wall xylans and arabinoxylans. *Journal of Histochemistry and Cytochemistry, 53*, 543-546.

Nishikubo, N., Awano, T., Banasiak, A., Bourquin, V., Ibatullin, F., Funada, R., Brumer, H., Teeri, T. T., Hatashi, T., Sundberg, B., & Mellerowicz, E. J. (2007). Xyloglucan endo-transglycosylase (XET) functions in gelatinous layers of tension wood fibres in poplar – A glimpse into the mechanism of the balancing act of trees. *Plant Cell Physiology, 48*, 843-855.

Northcote, D. H.; Davey, R.; & Lay, J. (1989). Use of antisera to localise callose, xylan and arabinogalactan in the cell plate, primary and secondary walls of plant cells. *Planta, 178*, 353-366.

Pinto, R., Amaral, A. L., Ferreira, E. C., Mota, M., Vilanova, M., Ruel, K., & Gama, M. (2008). Quantification of the CBD-FITC conjugates surface coating on cellulose fibres. *BMC Biotechnology, 8*, 1-7.

Putoczki, T. L., Gerrard, J. A., Butterfield, B. G., & Jackson, S. L. (2008). The distribution of un-esterified and methyl-esterified pectic polysaccharides in *Pinus radiata*. *IAWA Journal, 29*, 115-127.

 ISSN 0048 - 0134

Ruel, K., & Joseleau, J.-P. (1984). Use of enzyme-gold complexes for the ultrastructural localisation of hemicellulose in the plant cell wall. *Histochemistry, 81*, 573-580.

Ruel, K., Montiel, M.-D., Goujon, T., Jouanin, L.; Burlat, V., & Joseleau, J.-P. (2002). Interrelation between lignin deposition and polysaccharide matrices during the assembly of plant cell walls. *Plant Biology, 4*, 2-8.

Ruel, K., Chevalier-Billosta, V., Guillemin, F., Berrio Sierra, J., & Joseleau, J-P. (2006). The wood cell wall at the ultrastructural scale – Formation and topochemical organisation. *Maderas Ciencia y Technologia, 8*, 107-116.

Schmitt, U., Singh, A., Frankenstein, C., & Möller R. (2006). Cell wall modifications in woody stems induced by mechanical stress. *New Zealand Journal of Forestry Science, 36*, 72-86.

Taylor, J. G., Owen, T. P., Koonce, L. T., & Haigler, C. H. (1992). Dispersed lignin in tracheary elements treated with cellulose synthesis inhibitors provides evidence that molecules of the secondary cell wall mediate wall patterning. *The Plant Journal, 2(6)*, 959-970.

Taylor, N. G., Laurie, S., & Turner, S. R. (2000). Multiple cellulose synthase catalytic subunits are required for cellulose synthesis in *Arabidopsis*. *The Plant Cell, 12*, 2529-2539.

Vincken, J.-P., Schols, H. A., Oomen, R. J. F. J., McCann, M. C., Ulskov, P., Voragen, A. G. J., & Visser, R. G. F. (2003). If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. *Plant Physiology, 132*, 1781-1789.

Willats, W. G. T., Steele-King, C. G., McCartney, L., Orfila, C.; Marcus, S. E., & Knox, J. P. (2000). Making and using antibody probes to study plant cell walls. *Plant Physiology and Biochemistry, 38*, 27-36.

Wojtaszek, P., & Bolwell, G. P. (1995). Secondary cell-wall-specific glycoproteins from French bean hypocotyls. *Plant Physiology, 108*, 1001-1012.

Wyatt, R. E., Nagao, R. T., & Key, J. L. (1992). Patterns of soybean proline-rich protein gene expression. *Plant Cell, 4*, 99-110.

Ye, Z.-H., Song, Y.-R., Marcus, A., & Varner, J. E. (1991). Comparative localization of three classes of cell wall proteins. *The Pant Journal, 1*, 175-183.

Ye, Z.-H., & Varner, J. E. (1991). Tissue-specific expression of cell wall proteins in developing soybean tissues. *Plant Cell, 3*, 23-37.

Zhang, Y., Brown, G., Whetten, R., Loopstra, C. A., Neale, D., Kieliszewski, M. J., & Sederoff, R. R. (2003). An arabinogalactan protein associated with secondary cell wall formation in differentiating xylem of loblolly pine. *Plant Molecular Biology 52*, 91-102.

 ISSN 0048 - 0134