RESIN AND RESIN CANALS IN FAMILIES AND CLONES OF *PINUS RADIATA* (D. Don)

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Degree of Master of Forestry Science

By

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Abstract

Resin and resin canals are seen as defects in softwoods. The occurrence of these defects in appearance-grade radiata pine timbers causes devaluation. Living trees use resins and the resin canal networks as a defensive mechanism against pest and diseases by sealing wounds. This study determined the variation in resin quantity and resin canal features (density, percentage canal area, and canal size) between 2 year-old radiata pines grown from different genetic material. Out of an experiment for NZ radiata pine seed and clonal commercial deployment populations, 10 clones and 20 families were selected for the study. Near infrared (NIR) spectroscopy was used to estimate resin quantity whereas microscopy was used to assess resin canal features. Wood samples were heated to help gather resin at the cross-section followed by the collection of absorbance spectra with an NIR fibre optic probe. Wood sections (~20 μm thick) were imaged with a 2400 dpi flatbed scanner in polarised light. ImageJ software was used to analyse the images and to determine the resin canal features. Data were analysed with the R statistical software. Resin quantity obtained by NIR varied significantly ($p < 0.01$) between clones and families with average values of 2.71 and 3.68 (AU) respectively. Average canal density was 0.69 canal/mm^2 for clones and 1.53 canal/mm^2 for families ($p < 0.01$). Average percentage canal area was 1.13% for clones and 1.53% for families ($p < 0.01$). In both families and clones, resin canals were of homogenous size of approximately 0.02 mm^2. There were weak genetic correlations between resin quantity and other canal features ($r = 0.07$ with canal density, $r = 0.10$ with percentage canal area). Resin canal frequency was strongly correlated with percentage canal area ($r = 0.94$). Variability between clones and families was present for all variables and summarised by the coefficient of variation (CV). Resin quantity had a CV of 61% and 51% for clones and families respectively. Resin canal features had CVs ranging between 10% and 31% across the families and clones. Resin quantity determined by NIR across the families and clones had 4%

heritability, whereas the resin canal features had ~ 30%. The low heritability found for resin quantity could be due to the indirect NIR assessment. The resin features correlated favourably with modulus of elasticity and longitudinal shrinkage. The lesser the resin features the stiffer the wood and the lesser longitudinal shrinkage. Basic density and volumetric shrinkage had no correlation with resin features. These results suggest that, presently, radiata pine growers can identify genetic material that is more suited for high-value appearance-grade timber as well as maintaining improved wood properties (stiffness, density, growth, and form) in the markets.

1. General Introduction

1.1 Introduction

1.1.1 Brief Notes on Radiata Pine

Radiata pine (*Pinus radiata* D. Don) is the major species for plantation forestry in New Zealand. This conifer is versatile, grows fast, and produces timber of good characteristics (Uprichard, 2002). The species has good machining properties and it is easy to dry. Its wood can be treated with chemical preservatives to prolong the serviceable life of the wood. Lumber from the species can be used for interior and exterior purposes. Engineered wood products like plywood, medium-density fibre board (MDF), or laminated-veneer-lumber (LVL) are also produced from radiata pine. In addition, radiata pine is pulped and it is known to have good papermaking properties (Harris, 1991).

During the previous decades huge research commitments have been undertaken in New Zealand to enhance the understanding of the species' silviculture, management, genetic potential, and variability, as well as wood quality and utilisation. For many years to come radiata pine will maintain the potential of being the favourite species for commercial plantation in New Zealand. Therefore, further improvement of radiata pine will help to sustain the forestry sector's contribution to the national economy.

1.1.2 Tree Breeding

The main focus of tree breeding programmes is to develop varieties that will guarantee increased growth and yield, better wood traits (such as stiffness, density, and dimensional stability) and to enhance how a tree adapts to its environment (Burdon, Carson, & Shelbourne, 2008; Namkoong, Kang, & Brouard, 1988; White, Adams, & Neale, 2007). Forest tree breeding in New Zealand commenced in the 1950s with radiata pine being the

most widely bred species. In both New Zealand and Australia, the main objective of earlier radiata pine breeding programmes was to realise genetic gain in volume and stem form. Radiata pine breeding achievements have resulted in the reduction of the harvestable age by 10 years, on average, in New Zealand (Jayawickrama, 2001). Similarly, Australia has had about 15 years drop in harvestable age (from age 45 to 30 years of growth) (Ivković, Wu, McRae, & Powell, 2006); however, fast-grown plantation softwood species, including radiata pine, are prone to wood quality concerns. Of particular note, is that it has an inferior corewood and this forms a large percentage of the stem.

Usually, tree breeding programmes take decades because of long breeding cycles. In New Zealand, attempts to shorten the long breeding cycles for radiata pine include recent studies which investigated the possibility of selecting trees for wood quality analysis at very early ages (Apiolaza, Walker, Nair, & Butterfield, 2008; Chauhan et al., 2013; H. X. Wu, Powell, Yang, Ivković, & McRae, 2007). Namkoong et al. (1988) mentioned that a fundamental condition for a successful breeding programme is the existence of genetic variation in the trait of interest. Radiata pine meets such a basic condition with a vast between-tree genetic variation for several wood quality traits (Apiolaza et al., 2008).

Clonal and family forestry are the two main deployment strategies adapted over the years in New Zealand. Family forestry involves the large scale deployment of superior full-sibling families in operational plantation. In the 1980s New Zealand planting material for the national plantation programme in New Zealand was met by seeds from orchard farms (Burdon et al., 2008). Clonal forestry involves planting a few (10 – 50) genotypes that have shown their superiority in large scale clonal trials (White et al., 2007). Clonal forestry was predicted to be commercially viable, when the advantages in stem growth, form, disease resistance, and wood quality were taken into consideration (Burdon et al., 2008).

The advantages of clonal and family forestry deployment strategies, as explained by Burdon (1989) include:

- The possibilities of high selection intensity because only the few top ranked parents are considered.
- The elimination of pollen contamination, which minimises genetic gain.
- Complete prevention of inbreeding.
- Significant gains because specific combining abilities are secured, especially in cases where a large number of full-sibling families pass the genetic test.
- The operational deployment of interspecific hybrids between species is unreliable for inter-pollination in an open pollinated seed orchard.
- Allows flexibility and deployments are facilitated for specialised varieties focusing on specific products/markets change or adapting to unique environmental condition.
- Specific management practice such as to optimise silvicultural regimes for specific clones (Ahuja & Libby, 1993).

Moreover, there are two common propagation channels used to produce sufficient planting materials for operational forestation. The first method is to use controlled pollination (CP) to generate seedlings of full-sibling families. The second propagation method involves vegetative propagation of seedlings produced from CP seeds using techniques, such as tissue culture, somatic embryogenesis. The choice of propagation method depends on relative ease and cost. The major concern for clonal forestry is the lack of genetic diversity in typical plantations (White et al., 2007), which is worrying in the event of a calamity, such as pest infestation.

1.1.3 Heritability

Falconer and Mackay (1996) summarise heritability (h^2) as the percentage of phenotypic variance among individuals in a population which is resulting from an effect for the whole genotype, made up of the total number of additive, dominance, and epistatic effects. Heritability is frequently used by breeders to estimate how precise field trials are. It describes the response of genetic traits to selection (Piepho & Mohring, 2007). Poehlman (1979) explained that the heritability value is a way of estimating how a parameter is influenced by genetics and the environment. An h^2 value with a range between 0.6 - 1 means that genetic factors have a higher influence compared to environmental factors. A range of h^2 value estimates (0.06-0.62) has been reported for most conifer wood traits (Jayawickrama, 2001; Kumar, 2004; Lepoittevin et al., 2011). In mature *Pinus massoniana* from different sites in China, h^2 for resin yield was estimated between 0.13 and 0.58 with variability of about 21% (Zeng et al., 2013). In Sweden, Fries, Ericsson, and Gref (2000) estimated h^2 of 0.6, 0.3 to 0.8, 0.6 to 0.9 for resin acids, fatty acids, and sterols, respectively with a variability ranging between 30 to 80%. For resin content in *Pinus radiata* Cown, Young, and Burdon (1992) estimated a h^2 value of 0.5 whereas a h^2 value estimate of 0.4 was reported for resin canals in *Picea abies* (Hannrup et al., 2004). A recent study has found candidate genes responsible for resin canal formation in *Pinus taeda* and their influence on resin canal variability (Westbrook et al., 2015). The finding by these authors is an indication that resin canals in pines are partly under genetic control. In contrast, resin canals features may also change as a result of intensive silvicultural operation on tree and stand (Ananias et al., 2010).

1.2 Research Rationale

Resins and resin canals are defects in radiata pine wood especially for appearance-grade uses. Frequently, resin in radiata pine lumber is quite visible to the unaided eye making the

wood unappealing (Figure 1). Resin is also a sticky substance which can cause problems with paints on wood; however, in other softwood species such as slash pine (*Pinus elliottii*), resins are commercially tapped and used as a raw material for various chemical and pharmaceutical applications (Rodrigues & Fett-Neto, 2009). The formation of resin and resin canals were found to be controlled genetically as outlined above.

Figure 1: The cross-section of radiata pine wood with oil-looking resin.

Assessing the variability in resin features for radiata pine clones and families grown in New Zealand can improve the value of the radiata pine resource. The economic losses observed within the New Zealand forest industry as a result of the presence of resin in radiata pine could be reduced (McConchie, Cown, & Donaldson, 2008; Park & Parker, 1982).

At present, there is little information on variability in the amount of resin and resin canal features among the radiata pine families and clones grown in New Zealand. Findings could guide the selection of superior clones and families of radiata pine for further genetic improvement.

The aim of this study was to evaluate wood quality in terms of resin and resin canals features for families and clones of the New Zealand radiata pine production populations and to test if these variables were under genetic control.

1.3 Hypothesis and Research Questions

The hypothesis of this research:

i. The resin quantity and resin canal features (density, size and percentage [%] area) vary between families and clones of radiata pine and are partly under genetic control.

The research questions:

i. How does resin quantity correlate with resin canal features?
ii. Can the families and clones be ranked on the basis of resin quantity and canal features?

1.4 Specific Objectives

The specific objectives for this study were:

i. To estimate the relative resin quantity for families and clones using NIR spectrometry.
ii. To determine resin canals features for families and clones by microscopy.
iii. To test for variability in resin quantity and resin canal features for families and clones.
iv. To estimate the heritability value for resin quantity and resin canal features.
v. To rank the families and clones by their resin quantity and resin canals features.
vi. To determine the relationship between resin quantity and resin canals features as well as other wood properties.

2. Literature Review

2.1 Resin and Resin Canals in Softwood

Resin in softwood, commonly referred to as *oleoresin*, is a coloured, sticky, water insoluble, and complex mixture of organic substances. Its major components are fatty and resin acids (non-volatile in pines), esters of these acids, sterols, terpenes, and waxes (Bamber & Burley, 1983; Engstrom & Back, 1959). It is reported that resin in pines contains about 25% volatile compounds. Resins are a source for chemicals such as rosin and turpentine. In its technical term *resin* includes a variety of phenolic compounds such as flavonoids, lignans, and stilbenes (Sjöström, 1981). In the sapwood of radiata pine wood, resin constitutes about 1-2% of its dry weight (Bamber & Burley, 1983; Cown, Donaldson, & Downes, 2011), but, after heartwood formation, resin content increases as it is impregnated into dead tissues and can account for up to 25-30% of the weight of heartwood (Cown et al., 2011). In another study with *Pinus elliottii* and *Pinus taeda*, Larson, Kretschmann, Clark III, and Isebrands (2001) observed a similar trend of increased resin quantity with age. Extraction of resin from wood can be achieved by the use of organic solvents such as ethanol, acetone, and dichloromethane (Hillis, 1962).

Resins are different from heartwood extractives, which are deposited in wood while the secondary process of heartwood formation occurs at the sapwood-heartwood boundary (Hillis, 1962; Taylor, Gartner, & Morrell, 2002)

Resins in softwoods can occur, depending on species, either in individual parenchymatous cells or in specialised cells that form canals or ducts between tracheids. The specialised cells, termed epithelial cells, surround the canal which can exist in radial and axial direction within the wood.

Resin canals in radiata pine, as seen in Figure 2, are typically tubular, intercellular ducts and are oriented in both radial (horizontal) and axial (vertical) directions creating a complex network (Baas et al., 2004). The tubular structure is lined by a single layer of resin-secreting epithelial cells. A thin and unlignified primary cell wall of the epithelial cells is a feature of the genus *Pinus* (H. Wu & Hu, 1997). They produce resin that is released into the canals by a hydrostatic pressure generated within the canal network (Engstrom & Back, 1959). Among Pinaceae, genera such as *Abies* (firs), *Tsuga* (hemlocks), *Pseudolarix* (golden larch), and *Cedrus* (cedar) only develop axial resin canals in response to stress or external stimuli, whereas others, such as *Picea* (spruce), *Larix* (larch), *Pseudotsuga* (Douglas fir) and *Pinus* (pine) have resin canals widely scattered in their xylem. Fahn and Zamski (1970) additionally indicated that *Juniperus* (juniper) and *Cupressus* (cypress) possess no resin canals. Taxonomically, resin canals are important characteristics for softwoods identification (Bamber & Burley, 1983; H. Wu & Hu, 1997).

The axial resin canals in pines generally range from 40-200 μm in diameter (Baas et al., 2004; Bamber & Burley, 1983; Werker & Fahn, 1969); however, they could occasionally reach a diameter of 300 μm (Sjöström, 1981).

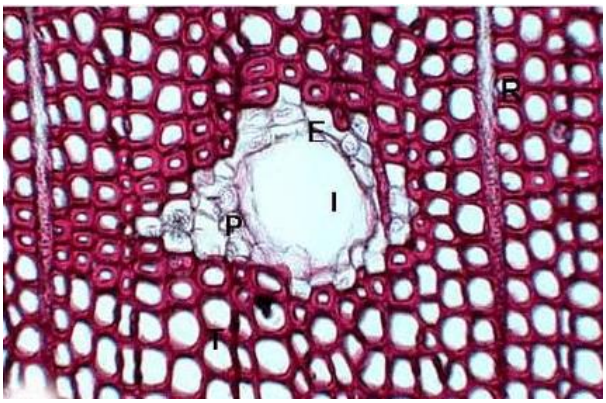

Figure 2: A resin canal in a cross-section of radiata pine. Resin canal (I), parenchyma cell (P), epithelial cell (E) (Boschiero & Tomazzello-Filho, 2012). It can be seen from the image that

unlignified epithelial cells have not been stained with Safranin in contrast to the tracheids with lignified cell wall.

2.2 Biological Benefits of Resin to Conifers

Trees are often subjected to physical, biological, and environmental stress, causing injuries to trees. Conifers have developed adaptive strategies that kill, repel, inhibit, or reduce attacks from invading herbivores and pathogens (Keeling & Bohlmann, 2006). Resin and resin canals form a defence centre and protect trees from the stress emanating from insect or fungal attack. Resin and resin canals also assist in the repair of mechanical wounds to the cambium through the activities of browsing animals or, nowadays, silvicultural operations (Butterfield, 2006). The presence of resins has been reported to improve the defence of pines against insect attacks (Ferrenberg, Kane, & Mitton, 2014; O'Neill, Aitken, King, & Alfaro, 2002; Trapp & Croteau, 2001). The genera *Abies, Tsuga, Cedrus* and *Pseudolarix*, which normally do not form resin canals, start to develop them during injury. Trees of regularly resin canal forming genera *Pinus, Picea*, *Larix*, and *Pseudotsuga* increase the frequency of their resin canals in order to produce more resin to heal wounds. Resin canals developed because of injury are referred to as traumatic resin canals and these can form resin pockets (large resin containing structures). Traumatic resin canals are also initiated when the cambium of conifers is ruptured as a result of wind sway, browsing by animals, or silvicultural operation, such as pruning (Clifton, 1969). In a European study with *Picea abies*, Temnerud (1997) outlined four groups of theories likely to cause resin pockets. They included cambial cell collapse due to wind, internal stress during growth, injury to the phloem and xylem layers because of insects and micro-organisms, and the combination of any of the three factors. In radiata pine, Cown et al. (2011) reported that resin pockets are universally present, ranging from low to high frequency. In New Zealand, Woollons, Manley, and Park (2008) concluded that

accelerated tree growth, stress associated with moisture and exposure play key roles when resin pockets in radiata pine are formed. It is interesting to note that, wood ants *(Formica paralugubris)* keep solidified conifer resin in their nest to avoid bacterial and fungal infection (Castella, Chapuisat, Moret, & Christe, 2008)

2.3 Resin Features as Wood Defects

Resins and resin canals exist in most softwoods and exhibit variation. Resin related features are classified as defects in timber and log grading. The resin features are important when assessing the potential of radiata pine wood for appearance-grade quality in New Zealand (Woollons et al., 2008). Mostly, pine wood fails to appeal to the eye due to the presence of resin (Walker, 2006) and results in significant loss of value for clearwood products. Significant loss of value caused by resin pockets in pruned radiata pine timber has also been reported by Park and Parker (1982).

Resin is known to dissolve paints and most finishes added to wood products for the purpose of increasing its value (Hoadley, 1998; Hse & Kuo, 1988; Williams, Jourdain, Daisey, & Springate, 2000) (Figure 4). Resinous pine boards can “bleed” extensively after treatment with a chemical preservative (Dawson, Kroese, Hong, & Lane, 2002).

Figure 3: Resin dissolving paint on a board (photo credit: Solid Wood Initiative, New Zealand).

Resins canals create indentations on painted wood boards interfering with their surface smoothness and brightness (McConchie et al., 2008), as can be seen in Figure 4. These indentations are visible after paint layers have been applied, especially with glossy finishes, for example in weather boards.

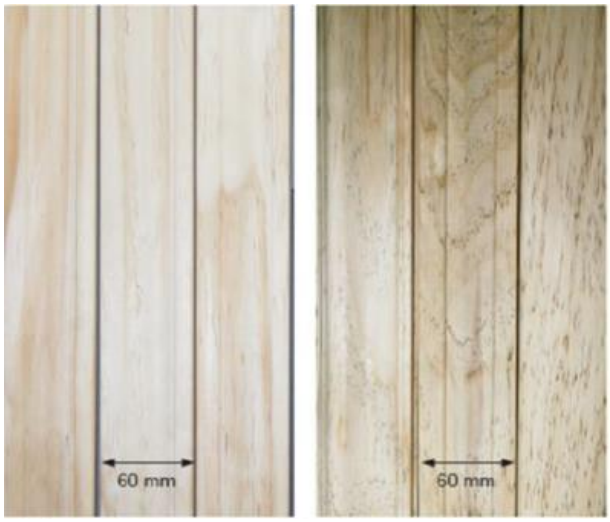

Figure 4: Variation in surface brightness between boards caused by resin canals. (photo credit: Solid Wood Initiative, New Zealand).

Resins are known to be the major cause of pitch problems during paper production (Uprichard, 2002). During pulping, resin canals and pockets readily rupture releasing the bulk of the stored resin into the pulping liquor. Later in the pulping process, the resin forms viscous lumps and accumulates on the pulping equipment and as well appears as oily specks in paper. The accumulated material, which melts when heated, is referred to as pitch and contains considerable amount of resin (Hillis & Sumimoto, 1989). The management of pitch problems through the use of chemicals results in increased production cost.

Resin in wood also adversely affects the pre-treatment of wood with preservatives (Ahmed, Sehlstedt-Persson, Karlsson, & Morén, 2012). Ahmed and colleagues observed that the number of longitudinal resin canals and the proportion of blocked canals contributed to the uneven distribution of preservatives. Equally, wood preservatives take advantage of resin canals to travel or flow into inner parts of solid wood. Knowledge of resin canals in wood will influence, for instance, how long to soak wood for effective preservation.

Resinous woods give off volatile organic compounds (VOCs) into the atmosphere (Thompson & Ingram, 2006) posing health and environmental concerns. In an enclosed drying system the discharge from drying radiata pine usually contains high chemical oxygen demand (COD) of 256-280 mg/L, high total organic carbon (TOC) of 56-69 mg/L, and a low pH of about 3.7 (Pang, 2012). McDonald, Dare, Gifford, Steward, and Riley (2002) mentioned α-pinene and β-pinene forming 90% of the kiln discharge. McDonald and colleagues, however, indicated the levels emitted are not harmful to the environment. Hydrocarbons of 2.21 g and traces of monoterpenes were reported to be emitted during the drying of ponderosa pine (Lavery & Milota, 2001). A good estimation of VOCs or kiln emissions during timber drying will be of importance as local bodies tighten their emission regulations.

2.4 Resin as a Resource

Globally, pines have been historically tapped commercially for resin to the benefit of farmers and national economies (Stanley, 1991; Wang, Calderon, & Carandang, 2006). During the kraft pulping process, volatile gases from resin are condensed to yield sulfate turpentine. Turpentine is an oily fraction mainly of monoterpenes and volatile sesquiterpenes and is used as solvent for special inks. Chemical and pharmaceutical industries also use turpentine (Boschiero & Tomazzello-Filho, 2012). Fats are saponified by the alkaline liquor which results in fatty and resin acids further conversion into their sodium salts. This is called rosin. Before the recovery of the inorganic pulping chemical, insoluble soaps are skimmed from the surface and acidified to yield tall oil rosin and fatty acids as by-products (Fengel & Wegener, 1984; Panda, 2013). Tall oil, also referred to as liquid rosin, is a yellow to black odorous liquid. Its chemical composition is a mixture of resin acids, terpenoids, fatty acids triglycerial oils, unsaponifiables, water, and ash (Johansson, 1982). Fatty and resin acids of spruce have been successfully converted into biodiesel (Demirbas, 2011), with that fatty acids being the most desirable portion of tall oil for biodiesel production.

The main acids in rosin from pines are abeitic and pimaric acid (Li, Luan, et al., 2012) Rosin is available as natural product and composed of 90% acidic and 10% neutral compounds (Zhang, 2012). Rosin is part of chewing gums and cosmetics, and as well, numerous pharmaceutical products (Kumar & Gupta, 2013; Yadav, Gidwani, & Vyas, 2016). The manufacture of glue rosin commonly used in the paper industry is heavily dependent on rosin as major raw material (Boschiero & Tomazzello-Filho, 2012).

The increasing potential of conifer resin has reinforced the need to sustain resin production. A number of studies investigated the genetic parameters for resin production among conifer species (Lepoittevin et al., 2011; Roberds et al., 2003). Li, Jiang, and Luan (2012) found significant variation for resin productivity among slash pine half-sibling

families in China. The authors also estimated heritability for resin production to be about 0.8. Similarly, a study with slash pine in the United States estimated a heritability value of 0.85 for oleoresin yield (Franklin, Taras, & Volkman, 1970). For *Pinus sylvestris*, heritability for resin acids was estimated to be approximately 0.6 (Fries et al., 2000). Knowledge of resin inheritance is needed to help control the trait and facilitate its inclusion into breeding programmes.

2.5 Variation in Resin and Resin Canals in Softwood.

A major concern in wood utilisation is the uniformity of any wood quality trait. Within a stem, resin quantity varies and can be of an irregular pattern (Sjöström, 1981). Sjöström noted, for example, that, heartwood in pines is more resinous than sapwood, whereas the opposite seems to be true for *Picea abies.*

Trees usually grow under different growth conditions and variation in resin canal features has been found in several studies (Boucher, Lavallée, & Mauffette, 2001; Carmona, Lajeunesse, & Johnson, 2011; Ferrenberg et al., 2014; Kolosova & Bohlmann, 2012; O'Neill et al., 2002). Wood properties including resin content and yield vary with species, site, age, and genetics (Davis, Jarvis, Parise, & Hofstetter, 2011; Li, Jiang, et al., 2012; Rodrigues & Fett-Neto, 2009; Sukarno, Hardiyanto, Marsoem, & Na'iem, 2015). Resin associated defects such as resin pocket can be typically influenced by environmental factors as reported for radiata pine by Woollons et al. (2008). Furthermore, a New Zealand study with 20-year-old half-sibling families of radiata pine revealed that resin content had at least 40% CV (Cown et al., 1992). The authors indicated that such a high CV value suggests the feasibility of genetic modification of radiata pine in relation to its resin content.

2.6 Brief Overview of Near-infrared (NIR) Spectroscopy

Near-infrared (NIR) is the electromagnetic spectrum in the range of 14300 to 4000 cm^{-1} (Osborne, Fearn, & Hindle, 1993); however, for NIR reflectance studies the most useful region for quantitative analysis remains the wavenumber range from 8333 to 4000 cm^{-1} (Norris, 1989). Osborne et al. (1993) note that spectra occurring in the NIR region consist largely of overtones and combination bands of the fundamental stretching vibration of O-H, N-H and C-H functional groups.

NIR spectroscopy for data collection is rapid, non-destructive, and an easy-to-use application for estimating chemical properties of organic material, such as moisture or protein content. The technology is efficient and inexpensive to assess a large number of samples with sizes ranging from milled particles to raw or engineered solid organic materials, including wood (Schimleck, 2008).

Spectroscopic signals are information-rich, resulting in complex data (Tsuchikawa, 2007). For wood and paper products, assignments of spectra bands to wood components (holocellulose, cellulose, hemicellulose, lignin, and extractives) have been summarised (Schwanninger, Rodrigues, & Fackler, 2011). There has been successful real-time prediction of resin and fatty acids in bio-refinery materials (Lestander, Samuelsson, & Sveriges, 2010). Extractive content estimations by NIR spectroscopy have been reported for Turkish pine (*Pinus brutia* Ten) (Üner, Karaman, Tanrıverdi, & Özdemir, 2011), Scots pine (*Pinus sylvestris*) (Holmgren, Bergström, Gref, & Ericsson, 1999) and maritime pine (*Pinus pinaster*) (Lepoittevin et al., 2011). NIR spectroscopy has also been explored to study how the flow of extractives in Scots pine relate to heat variation during drying (Nuopponen, Vuorinen, Jämsä, & Viitaniemi, 2003).

3. Methodology

3.1 Wood Material

The wood samples were sourced from 2 year-old radiata pine trees of known family and clone genotypes. These genotypes are part of two production populations for New Zealand tree crop plantations. Samples originated from a genetic trial set up in 2011 in a randomised complete block design at Harewood, Christchurch. The experiment was part of an earlier project (FRST-Compromised Wood Project) led by the NZ School of Forestry at the University of Canterbury (Apiolaza, 2014). The plot, at the time of planting, included 49 controlled pollinated families and 10 clones, with 30 replicates each. Three months after planting, the plants were leaned and tied at a 15^{o} angle for 21 months. This silvicultural manipulation separated compression wood from opposite wood (normal wood) present in the lower and upper sides of the young stems respectively. From the base of each tree 10 cm long bolts were cut, debarked, and sawn along the grain to separate compression and normal wood. Some of the normal wood samples are shown in Figure 5. Samples were labelled according to the experimental design, making sure trees were traceable to their genotypes. Drying of samples started at 60% relative humidity (RH) which corresponded to 12-13% moisture content (MC) and later in an oven at 103^{o} C. The final MC for wood samples was 0%. The dried samples were subjected to mechanical tests using protocols published by Chauhan et al. (2013). Later, all samples were stored indoors for 3 years. This research study considered only normal wood.

Figure 5: Wood samples available from the 2011 *Compromised Wood* programme.

3.2 Methods

3.2.1 Amount of Resin

3.2.1.1 Wood samples for NIR spectroscopy

Twenty out of 49 families in the genetic trial were selected by using a ranked set sampling method based on assessed wood density and stiffness (Apiolaza, 2014). In addition to these 20 all 10 clones in the trial were chosen. All available replicates for the 30 selected families and clones, ranging between 14 and 21, were used. The wood samples were heated in an oven at 105^{o} C in an attempt to move the resin in the wood pieces to their cross-sectional ends (Nuopponen et al., 2003). The result is shown in Figure 6. After 24 hours of heating, samples were collected from the oven and kept in a desiccator. This equilibrated the wood pieces to room temperature and avoided adsorption of atmospheric moisture.

Figure 6: Cross-sectional ends of samples showing resin (the oily looking dots) after heating in an oven at 105^{o} C for 24 h.

3.2.1.2 Determination of resin quantity by NIR

A Bruker Tensor 37 NIR spectrometer fitted with a fibre optic device (Model N-500, FT-NIR, Bruker Optics, Ettlingen Germany) was used to collect the spectra. OPUS Spectroscopy software (v 7.5, Bruker Optics, Ettlingen Germany) was used to acquire spectra and control the instrument. The samples were scanned in absorbance mode in the range between 9,000 and 4,000 cm^{-1}. Spectra data were recorded at 8 cm^{-1} intervals accumulating 10 scans per spectra. The probe of the device, with diameter 3.5 mm, was targeted at the cross-sectional ends of the dry wood samples. Six spectra, three from each cross-sectional end, were collected at random locations and subsequently averaged for each sample. NIR spectra were also collected from pure resin gathered from a mature radiata pine log and averaged.

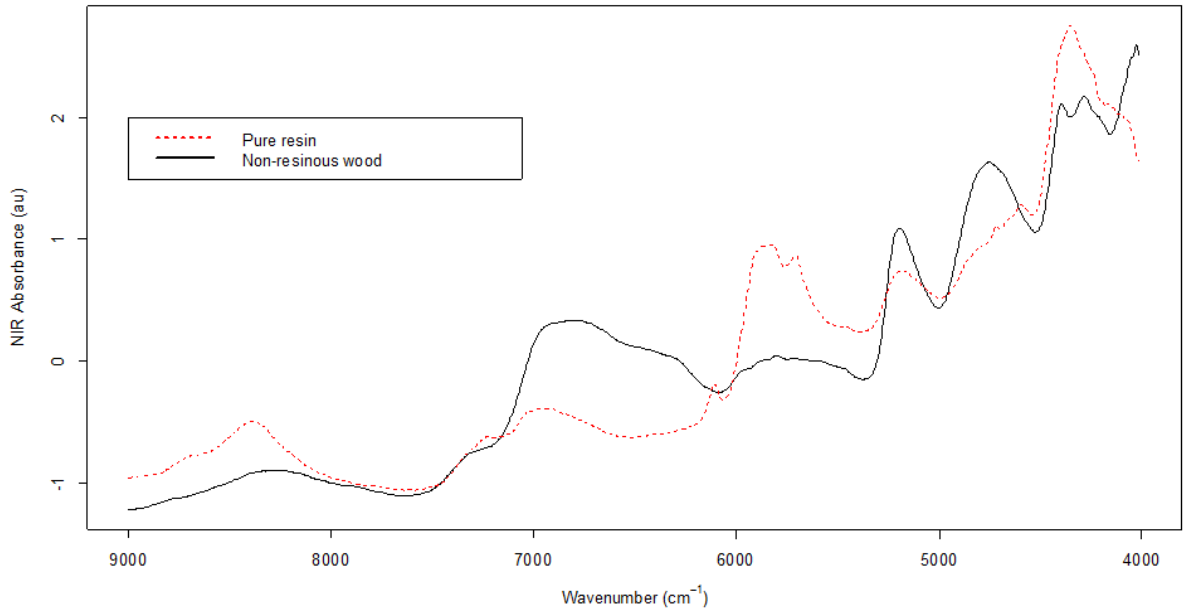

Figure 7: Standard Normal Variate (SNV) pre-processed spectra showing the difference between pure resin collected from radiata pine and a visually non-resinous study sample.

The R statistical software (R Core Team, 2016) was used to pre-process all spectra using the prospectr package (Stevens & Ramirez–Lopez, 2014). The spectra were pre-processed with the Standard Normal Variate (SNV) method which achieves baseline correction and normalisation (Figure 7). Subsequently the 1st derivative of the spectra (Figure 8) was calculated using the Savitzky-Golay algorithm to increase signal resolution (Savitzky & Golay, 1964). The spectra contain information regarding the chemical composition of the wood. Bands in the 9000 and 4000 cm^{-1} region have been assigned to specific chemical components of the wood (Barton, Himmelsbach, Duckworth, & Smith, 1992; Schwanninger et al., 2011). Both Barton and colleagues and Schwanninger and colleagues note that extractives, including resins, have been assigned to wavenumber 5974 cm^{-1} whereas cellulose is assigned to other wavenumbers including 5872 cm^{-1}. Both authors added that the band at wavenumber 5974 cm^{-1} is assigned to the 1st overtone of C_{ar}-H stretching vibrations. The

assigned bands for resin and cellulose are highlighted in Figure 8 and Figure 9, which displays the 1st derivative spectral of pure resin and non-resinous wood. Relative resin amount values were estimated as the difference in NIR absorbance at the wavenumbers 5974 and 5872 cm^{-1} for each spectrum. The second vertical line serves as an internal standard. It is the point of normalisation for cell wall mass.

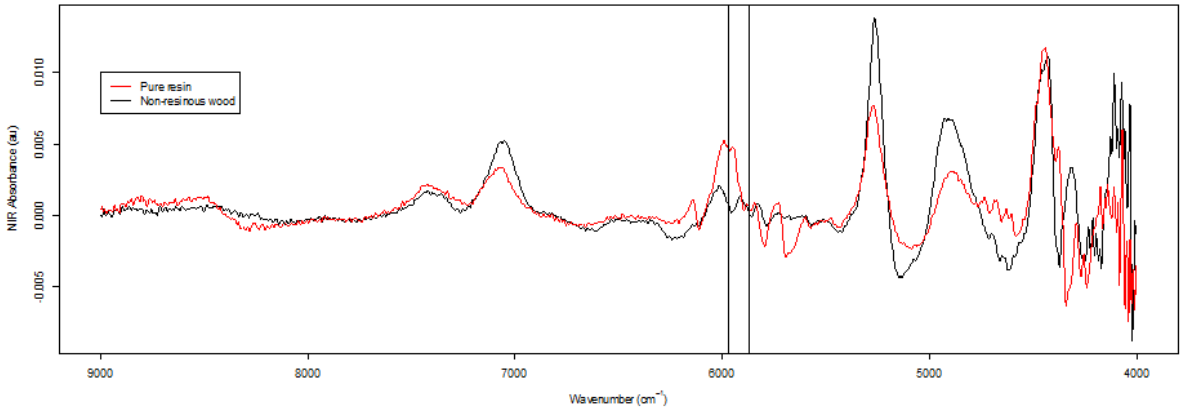

Figure 8: 1st derivative spectra from pure resin collected from a mature radiata pine log and a visually non-resinous study sample. Each spectrum represents the average of six spectra measured from the samples. A comparison at wavenumber 5974 cm^{-1} (band assigned to the extractive) shows the difference in absorbance between pure resin and radiata pine wood.

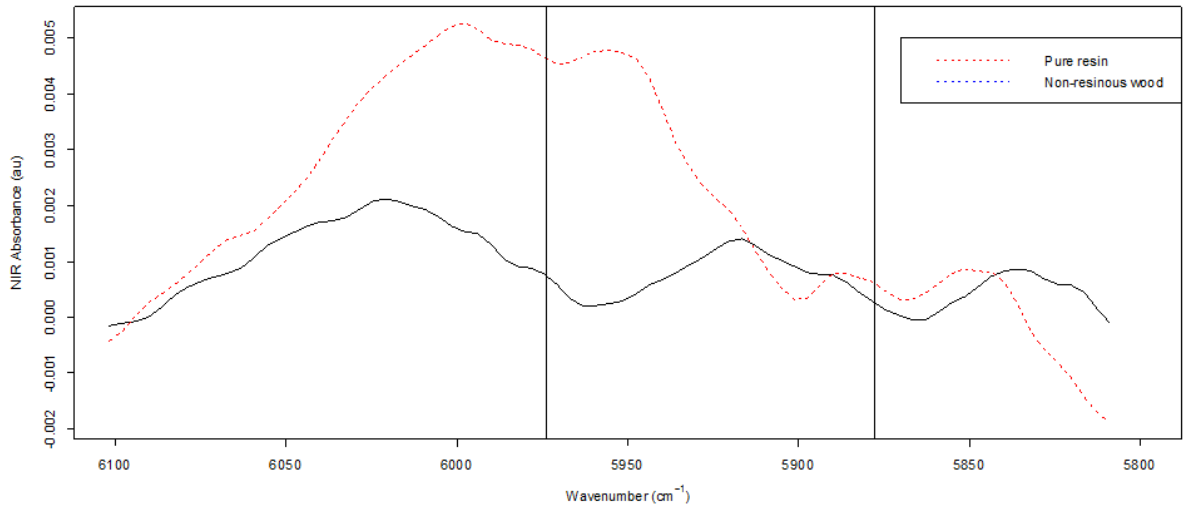

Figure 9: Peaks of spectra at the wavenumber range of interest (6100 to 5800 cm^{-1}). Wavenumber 5974 cm^{-1} (represented by first vertical line) shows the NIR absorbance difference between pure resin and visually non-resinous radiata pine.

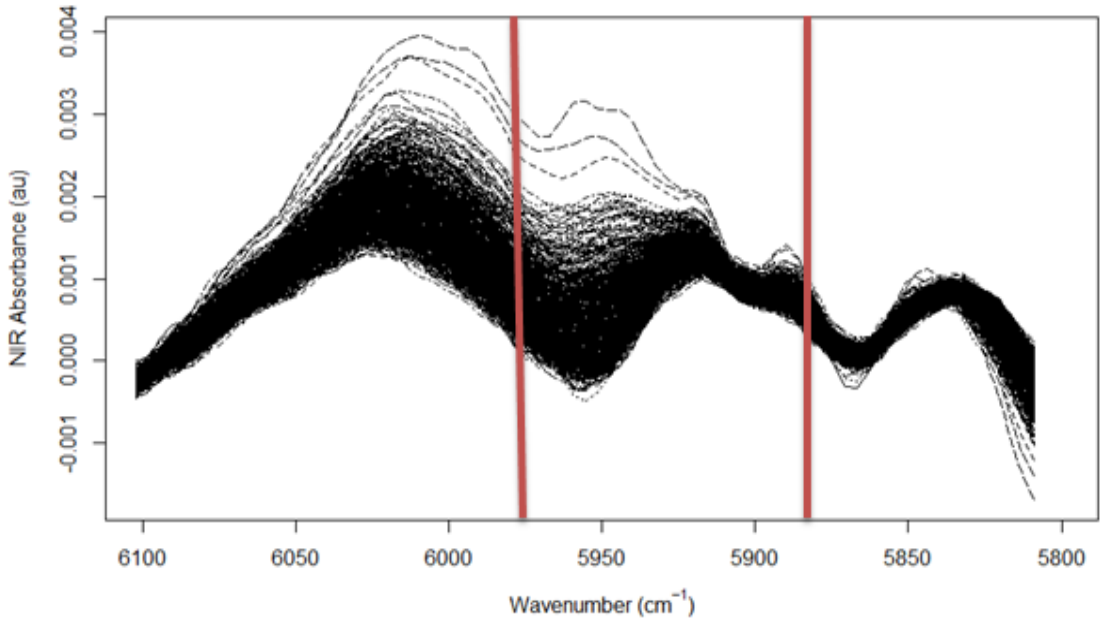

Figure 10: Showing the variation in NIR absorbance for all spectra at the wavenumber range of interest.

The wavenumber range of interest (6100 to 5800 cm^{-1}) for spectra from all study samples were averaged and pre-processed using SNV and 1st derivative (Figure 10). Also highlighted are the wavenumbers assigned to resin (5974 cm^{-1}) and cellulose (5872 cm^{-1}). It can be seen that spectra vary in intensity at wavenumber 5974 cm^{-1}. This is because of the variation in amount of resin present in the study samples. In contrast, spectra at wavenumber 5872 cm^{-1} are of consistent intensity providing an internal standard for the cell wall material.

3.2.2 Resin Canals

3.2.2.1 Sampling and preparation for microscopy

Ten replicates were randomly selected for each of the 20 families and 10 clones under study. The experiment, which focused on resin canals, did not consider all replicates used for NIR measurement due to the workload involved.

Wood pieces of 2 cm were cut from each of the 10 cm wood samples and placed into a labelled plastic vial containing water. The samples were then placed in an oven at 60^{o} C for 24 hours to soften the wood. The water in the vials was then replaced with a 1:1 solution of glycerol and ethanol (Figure 11) to further soften and preserve the wood samples before sectioning with a microtome (Schweingruber, 2007; Thomas, Ingerfeld, Nair, Chauhan, & Collings, 2013).

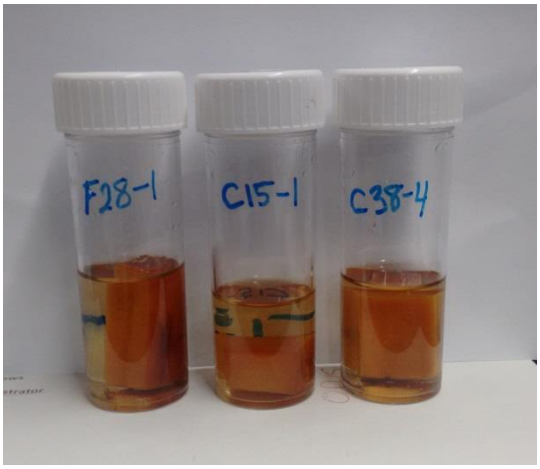

Figure 11: Vials containing wood samples in a 1:1 solution of glycerol and ethanol.

3.2.2.2 Measurements of resin canal characteristics

Sections ranging from 20 – 60 µm in thickness were cut from the cross-sectional face of the softened samples using a microtome (HM 400, Microm, Walldorf, Germany). The thin sections were brush picked carefully into a petri-dish containing water. After observing the sections under a compound microscope (Eclipse 80i with a DS-Ri1 camera, Nikon), best section for each sample was mounted on a labelled specimen slide (26 mm x 76 mm) using a drop of glycerol (Figure 12).

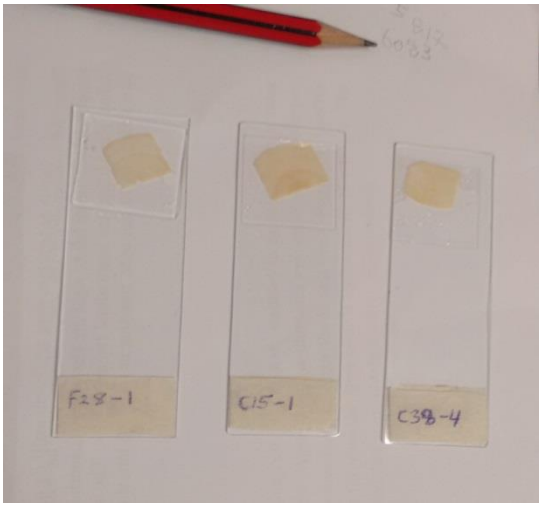

Figure 12*:* Wood sections mounted on slides.

The mounted sections were scanned in colour at 2400 dpi using a flatbed scanner (Epson Perfection V700) with linear polarised light. This was achieved by putting one sheet of linear polarising film under the prepared slide and a second polarising film, oriented at right angle, on top. Images scanned with polarisers have their backgrounds eliminated. The birefringent secondary cell walls in wood appear bright against the dark background; however, only a primary non-birefringent cell wall exists for parenchyma cells surrounding resin canals. In effect, the parenchyma cells appear dark, which makes the resin canals clearly distinguishable. This can be seen in Figure 13. Images from the scanned sections were uploaded into the ImageJ software (Abràmoff, Magalhães, & Ram, 2004) for further processing.

Figure 13: Image of a scanned wood section in polarised light displaying resin canals as black dots.

Figure 14: Image of a wood section in 8-bit format with threshold effect. These image enhancing features were applied in ImageJ software. Resin canals can be seen as black dots.

In the ImageJ software, macros were written to determine;

1. The area (mm^2) of the wood sections
2. The number of resin canals within the section
3. The average resin canal size (mm^2) for each section

Other tools in the ImageJ software, such as the *multiselect* and the *wand*, were also used to confirm the automated macro system. The macro set the appropriate image type and threshold limits (Figure 14). A total of 300 images (10 replicates from each of the 20 families and 10 clones) were used for anatomical measurements of resin canals.

The data were used to calculate the resin canal density and the relative resin canal area.

1. Resin canal density = resin canal count / area of section
2. % resin canal area = (average canal size*number of canals) / area of section

3.3 Statistical Analysis

The R statistical software (R Core Team, 2016) was used for data analysis. The packages, *lmerTest* and *lme4* in R, were used to calculate the variable means and to test for differences in means for the planting material types. For each variable, box plots were used to compare groups across families and clones. The CVs for material types were determined and compared to establish variability that exists within them. Furthermore, all variables were ranked using the Spearman's rank-order correlation method. Resin quantity was correlated against other resin canal features in their ranked states. The degree of genetic control (heritability) and genetic correlation were determined by adopting the protocols used by Apiolaza (2014).

4. Results and Discussion

4.1 Resin Quantity and Resin Canal Features

At the time of this study, all samples were sound without deterioration. Resin canals existed in all of the 2 year-old wood samples indicating the presence of resin. The estimated values for the variables were comparable between clones and families. The summary statistics for resin quantity, canal density, percentage canal area and canal size irrespective of the genetic material are presented in Table 1.

Table 1: Summary statistics for resin quantity and resin canal features in wood from 2 year-old radiata pine.

Parameter	Mean	Confidence Interval (95%)		Coefficient of variation (CV %)
		Lower	Upper	
Resin quantity (au)	3.37	3.22	3.52	55
Canal density (canal/mm^2)	0.83	0.80	0.86	31
% canal area	1.40	1.35	1.45	32
Canal size (mm^2)	0.01687	0.01666	0.01708	11

Comparatively, resin quantity estimated by NIR had the greatest variability (CV = 55%) whereas canal size had the least variability (CV = 11%). The latter suggests that resin canals were of homogeneous size. Canal density and percentage canal area had similar variability of ~ 30%. The arbitrary unit (au) assigned to resin quantity in this study, can be used to compare the planting materials in terms of their relative resin quantity. It is important to clarify that the resin quantities estimates by NIR were not validated with actual resin quantity values

obtained through chemical extraction from milled wood samples and therefore making values inaccurate.

In general for *Pinus radiata*, it has been estimated that the sapwood contains about 1-2% of the dry weight of resin (Bamber & Burley, 1983; Cown et al., 2011); however, at levels around the 2%, resin becomes a natural defect affecting pulp, timber, and veneer. As the young radiata pine grows, the mean values for resin quantity may increase with age as reported for *Pinus ponderosa* (Hood & Sala, 2015) and *Pinus radiata* (Cown et al., 2011). Although the study samples were 2 year-old, the estimated mean values (Table 1) for resin canal density and size agreed with estimates for the same variables (canal density range = 1.22 - 1.43 canal/mm^2, canal size range = 0.013 - 0.018 mm^2) in seedlings of *Picea sitchensis* and *Picea glauca* (O'Neill et al., 2002) and mature *Pinus radiata* (0.5 - 1.5 canal/mm^2, 0.015 - 0.025 mm^2), *Pinus caribaea* (0.27 - 0.66 canal/mm^2, 0.015 – 0.021 mm^2) (Ananias et al., 2010; Boschiero & Tomazzello-Filho, 2012).

Table 2 is a summary statistic for variables separated by material type. For all variables, full-sibling families had a higher mean than the clones. These differences were highly significant except for canal size. Figure 15 (diagonal) contains box plots of the studied variables for the young stems. Estimated variable mean and CVs for each family and clone are reported in Appendices A – D. In addition, ANOVA was used to test how the variable means statistically differ between families and clones. Those findings are reported in Appendices E and F.

Table 2: Summary statistic for resin quantity and resin canal features for 2 year-old radiata pine clones and families.

Parameter	Planting material	Mean	Confidence Interval		P-value of the differences between means	Coefficient of Variation (CV %)
			Lower	Upper		
Resin	Clone	2.71	2.48	2.95	2.18e-09	61.1
quantity (au)	Family	3.68	3.50	3.86		50.7
Canal density	Clone	0.69	0.65	0.73	4.73e-12	31.4
(canal/mm^2)	Family	0.90	0.87	0.94		27.8
% canal area	Clone	1.13	1.06	1.20	1.95e-14	30.7
	Family	1.53	1.47	1.59		28.3
Canal size	Clone	0.0165	0.0161	0.0169	0.0186	11.9
(mm^2)	Family	0.0170	0.0168	0.0173		10.1

The CV percentage was used to express the phenotypic variability within variables for the 2 year-old trees. For all the variables, clones had a higher variability than families. This shows that the clones were more different from each other as compared to similarity seen in the samples from the families. The rapid but coarse assessment of resin quantity by NIR is likely to have contributed to the wider variability for this trait. The fewer resin canals

observed for clones is consistent with the relatively lower resin quantity estimated associated because the canal size was uniform across study samples.

Variability can be caused either by genetic, environmental, or the interaction of both effects. The planting materials for this study were grown at a single location (Harewood, Christchurch), which provided homogenous growth conditions, thus minimising variation caused by the environment. This implies that the variability observed from this study should be attributed mainly to genetic factors among the planting materials. The scatterplot matrix in Figure 15 shows both the boxplot comparing variable means families and clones (diagonal) and relationship between variables (off diagonal). The canal density had a strong positive correlation with the percentage canal area for both, families and clones. Resin content had moderate positive correlation with canal density and percentage canal area. Canal size had no relationship with other variables.

The resin quantity in radiata, loblolly, and slash pine has been reported to vary genetically (Blanche, Lorio, Sommers, Hodges, & Nebeker, 1992; Cown et al., 1992; Li, Luan, et al., 2012; Roberds et al., 2003). Furthermore, factors such as tree age, season, tree growth, wounding and disturbance have been identified to cause variation of resin quantity in ponderosa and loblolly pine (Hood & Sala, 2015; Novick, Katul, McCarthy, Oren, & Sveriges, 2012; Sjöström, 1981). Similarly, variation in resin canal features have been suggested to be partly under genetic control (Rosner & Hannrup, 2004) and partly under environmental control (Rigling, Brühlhart, Bräker, Forster, & Schweingruber, 2003). Several studies have shown that softwood trees of same species but of different genotype vary in their defence against pests (insects) and diseases. Anatomical variation in xylem tissues and cells such as resin canals have been identified as an additional factor to resin content (Ferrenberg et al., 2014; Keeling & Bohlmann, 2006; Lenoir, Bengtsson, Persson, & Sveriges, 2003; Mottet, DeBlois, & Perron, 2015; Trapp & Croteau, 2001). In practice, the variability in resin

and resin canal features for radiata pine could be useful for selection of trees with superior quality (Zobel & Jett, 1995).

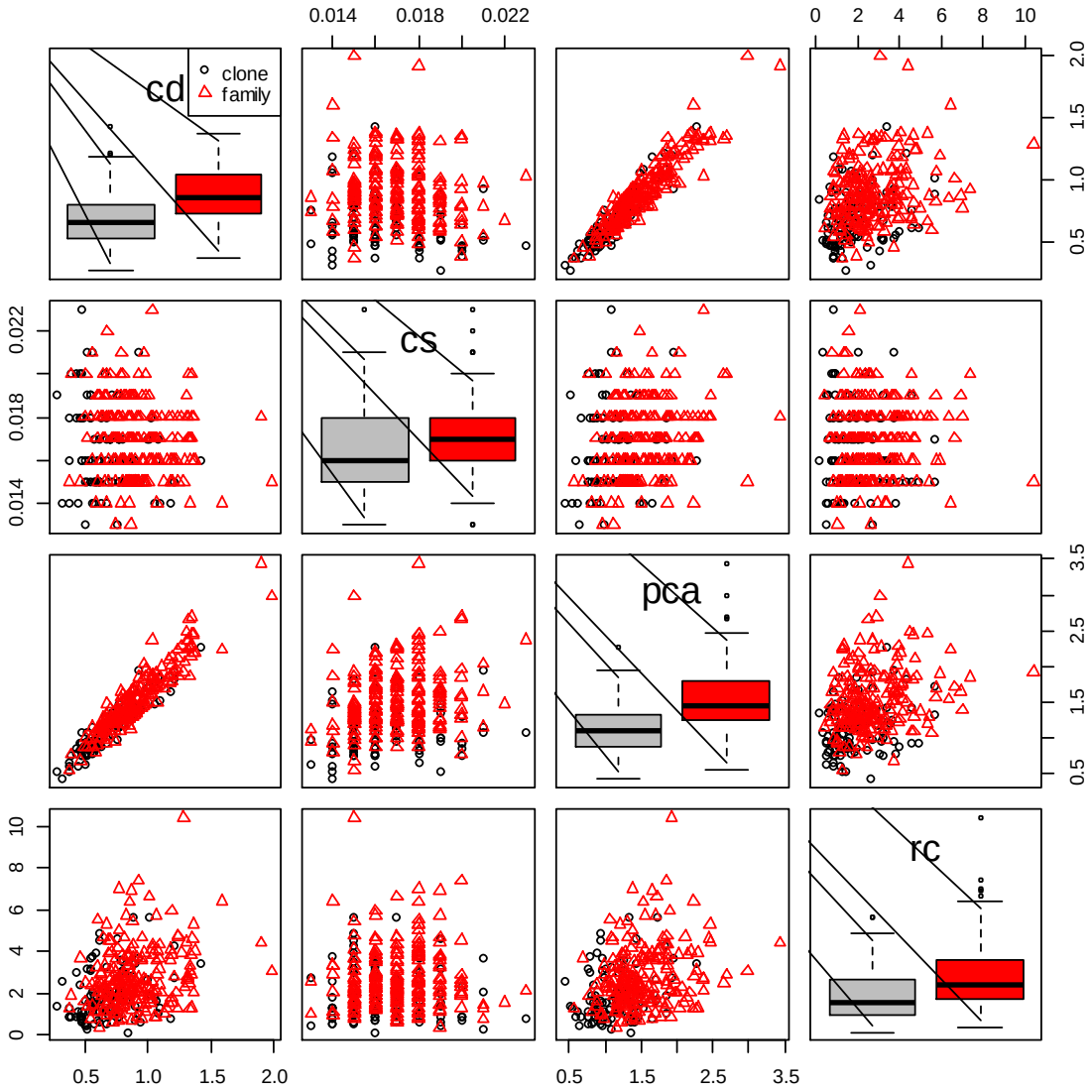

Figure 15: Scatterplot matrix for resin and resin canal features for 2 year-old radiata pine clones (black) and families (red). Canal density (cd), canal size (cs), percentage canal area (pca), resin content (rc).

The data suggest that, in this study radiata pine grown from clonal material will have fewer resin related defects. Wood products from family materials may be comparatively more resinous and have more associated defects like resin bleeding to dissolve paints and finishes (Dawson et al., 2002; Hoadley, 1998). Resin trapped in canals can hinder the flow of moisture in wood during drying and the flow of preservatives during treatments. Unblocked resin canals, however, serve as channels for chemicals permeating to the inner portions of a wood piece (Ahmed et al., 2012). In effect, higher resin canal density in radiata pine may not benefit appearance-grade products but can be an advantage during preservative treatments and timber drying (Cŏté, 1963; Flynn, 2007; Keey, Langrish, & Walker, 2012).

The pitch problem can be more pronounced when using family material for kraft pulping (Uprichard, 2002). Similarly, clonal materials that possess less resin can suggest that emissions from their kiln drying operations will be reduced. The emission of VOCs during kiln drying should not be overlooked as local regulations on the environment get tightened (Pang, 2012).

In contrast, the family materials will be preferred if this present radiata pine population are to be considered for commercial resin tapping (Boschiero & Tomazzello-Filho, 2012). This alternative industry supports individual, corporate and national economies (Stanley, 1991; Wang et al., 2006). For decades, natural wood resin serves as a resource in the chemical and pharmaceutical industry where it is used for numerous applications (Yadav et al., 2016).

Clifton (1969) and Cown (1973) each mentioned that stems with higher resin canal frequencies are likely to have a higher incidence of resin related defects such as resin pockets and blemishes during plant growth. Temnerud suggested that cambial cells are likely to collapse due to wind and internal stress (Temnerud, 1997). Varied resin canal density across

the genetic material means that indentations created on wood boards will vary, and therefore the smoothness and brightness of the boards' surface will also vary (McConchie et al., 2008). Clonal materials may be able to guarantee smooth and bright surfaces as opposed to family materials. Practically, the influence of canal size on the utilisation of the wood may not vary significantly between clones and family. The means estimated for canal size across families and clones (0.017 mm^2) showed little variability.

4.2 Heritability and Genetic Control

The level of genetic control for any wood trait determines success in a breeding programme. The study has revealed that variability shown by the parameters were partly under genetic control. Table 3 lists the heritability (h^2) values estimated for the resin quantity, canal density and percentage canal area.

Table 3: Mean and 95% confidence intervals, in parenthesis, for posterior distributions for heritability (diagonal) and genetic correlations (off diagonal) of resin and resin canal features.

Parameter	Resin quantity by NIR	Canal density	% canal area
Resin quantity by NIR	0.04 (0.01, 0.12)		
Canal density	0.07 (-0.33, 0.53)	0.31 (0.21, 0.45)	
% canal area	0.10 (-0.34, 0.61)	0.68 (0.49, 0.83)	0.29 (0.17, 0.45)

Zobel and Jett (1995) have commented that genetic control for most wood variables ranges between moderate to strong. Apiolaza, Chauhan, and Walker (2011) estimated low to

moderate h^2 values (ranging from 0.15-0.38) for wood quality traits of young (age ~ 2 years) radiata pines. For mature *Pinus radiata*, Cown et al. (1992) estimated a h^2 value of 0.5 for resin quantity. Heritability for resin yield in *Pinus massoniana* in China was estimated between 0.13 and 0.58 (Zeng et al., 2013). For *Pinus sylvestris* in Sweden, Fries et al. (2000) estimated h^2 of 0.6, 0.3 to 0.8 and 0.6 to 0.9 for resin acids, fatty acids, and sterols respectively. The study finding on h^2 value for resin quantity (0.04) is not comparable to earlier reports, and it might be attributed to the inaccuracy of assessing resin quantity by NIR.

In contrast, the h^2 values estimated for canal density and percentage canal size (approximately 0.3) are in agreement with and h^2 reported for other wood traits for radiata pine (Apiolaza, 2014; Apiolaza et al., 2011). The reported h^2 for resin canal density and percentage canal area are higher than h^2 estimated for radiata pine growth traits but lower than h^2 values estimated for other wood properties, including basic density (Apiolaza, 2014; Kumar, Burdon, & Stovold, 2008). In Norway spruce (*Picea abies*), Hannrup et al. (2004) reported a h^2 value of 0.41 for resin canal density.

It can be inferred from the study findings that breeding for resin canal density or percentage canal area would be as feasible as for other wood variables like stiffness and density. A reduction of the number of resin canals in radiata pine has been suggested to minimise the incidence of resin related defects in its wood (Cown, 1973; Cown et al., 2011; McConchie et al., 2008). In effect, timber will be more likely to qualify for appearance-grade and maintain its market value.

Additionally presented in Table 3 are the genetic correlation coefficients for the variables. Resin quantity correlated only weakly with canal density and percentage canal area (0.07 and 0.10 respectively). This means that the resin quantity by NIR for young radiata pine wood and its resin canals features (excluding canal size) are genetically independent variables. This

observation might be different if resin quantity had been assessed by solvent extraction of milled wood. In a study with radiata pine in Chile, Ananias et al. (2010) found a weak relationship between resin canals occurrence and external resin bleeding. This finding is however uncertain because they studied only six trees.

In contrast, canal density correlated strongly and positively with percentage canal area with a narrower 95% credible interval. This means that a breeding objective to reduce resin canal density will automatically reduce the percentage canal area and vice versa. This is not surprising given the homogeneous size of the resin canals.

4.3 Ranking of Clones and Families

Ranking of the study samples for individual variables was feasible. The estimated values for the variables were orderly ranked using box plots for all 10 clones and 20 families of radiata pine. For resin quantity, c-B had the overall least and was therefore the best material. The worst clone (c-C) was in the middle of the ranking level, while families with low resin quantity could still be identified (Figure 16).

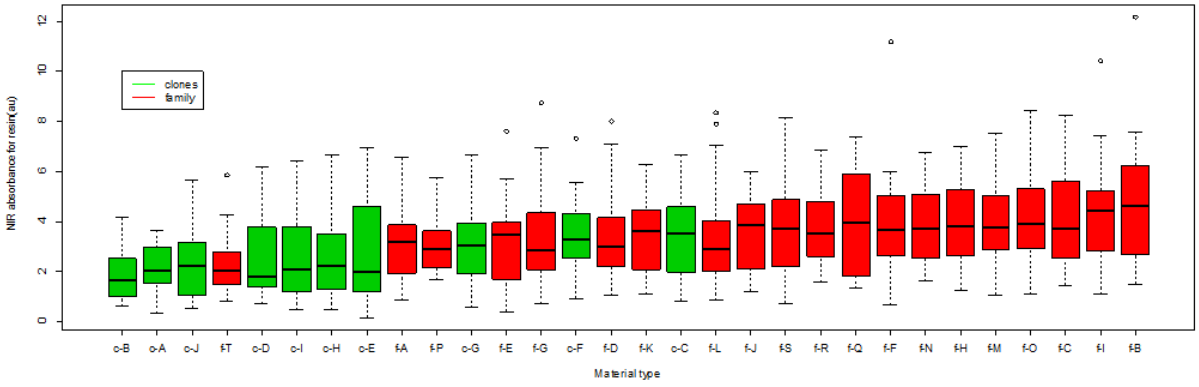

Figure 16: Box plots (ranked by mean) for resin quantity across clones and families of 2 year-old radiata pine.

For resin canal density, c-B was the best material (least number of canals per mm^2). The worst clone (c-J) was close to the 3rd quarter position whereas the best family (f-E) was at 1st quarter position (Figure 17). For percentage canal area, c-B was also the best (least percentage canal area) material (Figure 18). The worst clone (c-A) was in the middle of the pack, while families with lower percentage canal area could still be identified.

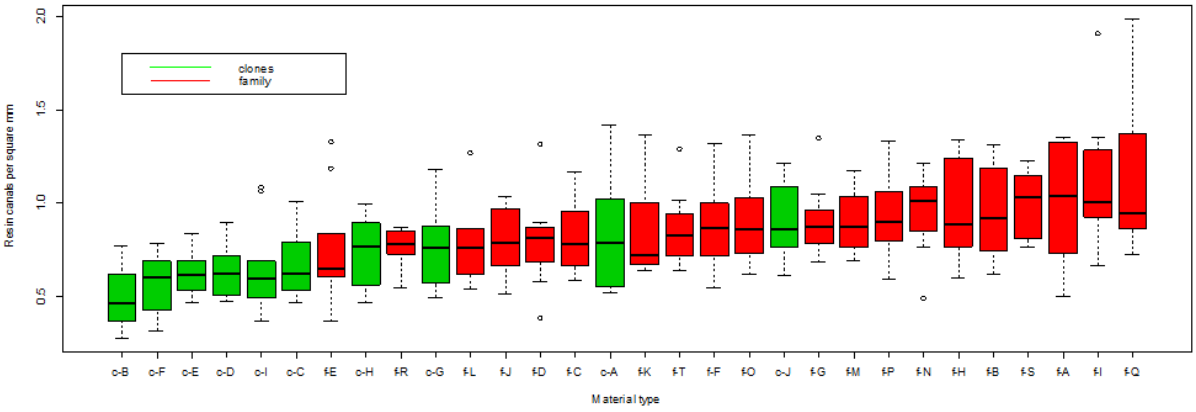

Figure 17: Box plots (ranked by mean) for resin canal density across clones and families of 2 year-old radiata pine.

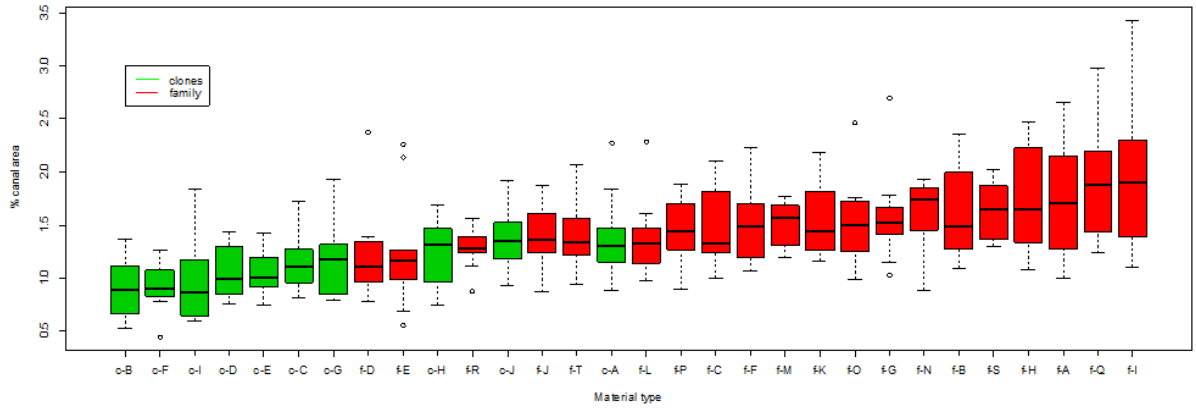

Figure 18: Box plots (ranked by mean) for percentage resin canal area across clones and families of 2 year-old radiata pine.

For canal size, there were overlaps for few materials because their estimated means were identical. c-I and f-D had comparatively the smallest canal sizes whereas c-B and f-K had the largest for clone and family genotype respectively (

Figure 19); however, the variability between them is small and likely not of importance.

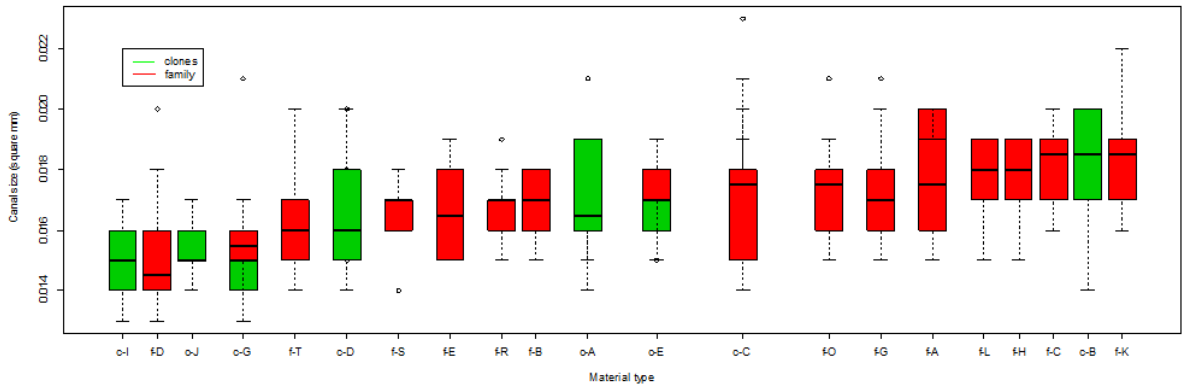

Figure 19: Box plots (ranked by mean) for resin canal size across clones and families of 2 year-old radiata pine.

The ranking of materials by different traits can be used to select the material that maximises genetic gain. The percentage genetic gain for resin quantity and canal density for mass selection is given in Table 4. This was estimated using the following formula;

$$\%\ genetic\ gain = h^2 * \frac{difference\ in\ means\ (population\ mean - mean\ for\ selected\ best)}{population\ mean}$$

For all variables, the mean for selected best (e.g. top 10%) within each of the families and clones was achieved by first sorting all individual values in increasing order across the clones and families. Afterwards, only the top 10% of each clones and families were considered in the calculation of a new mean. The population means are the respective variable means given

in Table 2. When compared, genetic gain for resin canal features was better than that of resin quantity.

Without taking into consideration age – age correlation of variables, the findings in this study will face concerns about uncertainty. Questions as to whether the values for the variables will remain significantly unchanged with age cannot be confidently answered; however, the study by Zeng et al. (2013) on the different ages of *Pinus massoniana* did find a fairly stable age – age genetic correlation for resin yield capacity. Out of the three sites in Zeng and colleagues study, only one presented an inconsistent age – age genetic correlation until age 11 when its results agreed with the results from the other two sites.

Table 4: Percentage genetic gain for resin features for 2 year-old radiata pine.

Parameter	Material type	Top 10% of genotype	Top 25% of genotype	Top 50% of genotype
Resin quantity	Clone	2.95	2.58	1.85
	Family	2.71	2.17	1.63
Canal density	Clone	13.04	10.14	7.24
	Family	14.44	11.11	8.89
% canal area	Clone	12.83	10.00	6.93
	Family	11.37	8.72	6.26

4.4 Correlation between Resin Features and Physical Properties

The association between resin features was discussed in section 4.2. In addition, selected physical properties such as basic density, modulus of elasticity, volumetric, and longitudinal shrinkage from the same study samples (Apiolaza, 2014) were correlated against the resin quantity by NIR and resin canal features. The correlation coefficients are provided in Table 5.

Table 5: Correlation coefficients (p-value in parentheses) for resin features against physical properties of 2 year-old radiata pine wood.

Parameter	Resin quantity	Canal density	% canal area	Canal size
Basic density	0.01 (0.81)	0.02 (0.74)	0.03 (0.60)	0.05 (0.36)
Modulus of elasticity (MoE)	**-0.23 (<0.00)**	**-0.29 (<0.00)**	**-0.32 (<0.00)**	-0.09 (0.11)
Volumetric shrinkage	0.05(0.43)	0.07 (0.23)	0.08 (0.15)	0.01 (0.82)
Longitudinal shrinkage	**0.12 (0.04)**	**0.21(<0.00)**	**0.25 (<0.00)**	0.07 (0.26)

Basic density was independent of the resin features. The sapwood of radiata pine contains approximately 1.5% dry weight of resin (Bamber & Burley, 1983; Cown et al., 2011). Such a low percentage mass does not significantly influence basic density. It could be inferred from this study that, breeding to influence any of the resin features studied may not affect the basic density for the 2 year-old radiata pine. The same can be said for volumetric shrinkage.

Interestingly, the modulus of elasticity had both a moderate and a negative correlation with resin features except canal size which was weak. This suggests that an increase in the modulus of elasticity will result in a decrease in resin features. In contrast, longitudinal shrinkage had a moderate and a positive correlation with resin features but not with canal size, and therefore a decrease in resin features will result in a decrease in longitudinal shrinkage. In both cases this is a favourable correlation because a high modulus of elasticity and low longitudinal shrinkage are the desired traits for *Pinus radiata* (Apiolaza, 2014).

The lesser resin canal density estimated for clones (Figure 15) can also be partly attributed to an unintentional breeding effect. It is worth mentioning that the clones were bred for growth and stiffness whereas the families were bred for growth and density. Hence the results indicating that clones have less resin canal features as compared to families. While stiffness has a negative correlation to resin features, density has no correlation to resin features.

5. Conclusion

Across the families and clones, resin quantity by NIR had the greatest variability (CV=55%) whereas canal size had the least (CV=11%). The latter indicates that resin canals are of fixed size in radiata pine. Estimated mean for canal density was 0.83 canal/mm^2, percentage canal area was 1.4 (%) and canal size was 0.01687 mm^2. Means estimated for all variables were higher in families than in clones. For instance, resin quantity for families was 3.65 (au) whereas in clones it was 2.71 (au) with a p-value of 2.18e-09. Interestingly for all the variables, clones had greater variability than families.

The study found that canal density and percentage canal area were under moderate genetic control with heritabilities (h^2) ranging from 0.21 to 0.45 and 0.17 to 0.45, respectively. Resin quantity was under weak genetic control (h^2=0.04). Contrary to this finding h^2 of 0.5 was reported for resin quantity estimated by chemical extraction from milled samples (Cown, 1973). It is likely that the low heritability and the high variability for resin quantity are due to a high error margin in the NIR method used in this study. There was a high genetic correlation between canal density and percentage canal area (0.68) and this was driven by the homogeneous resin canal size. Genetic correlation between resin quantity and canal features were very weak (<0.1), again probably due to the NIR technique used for assessing resin quantity.

The families and clones were ranked by their means for all variables. Clone c-B had the lowest estimated mean for all variables. There was moderate and positive correlation between resin quantity and canal density and percentage canal area (r = 0.33). Canal density correlated strongly positive with percentage canal area (r = 0.94). There was weak correlation between canal size and resin quantity and canal density (r < 0.3).

Resin features were correlated with basic density, modulus of elasticity, volumetric, and longitudinal shrinkages. Basic density and volumetric shrinkages had very weak correlations with resin features (correlation coefficient <0.1). Longitudinal shrinkage had moderate and positive correlations with resin features (r = 0.12, 0.21 and 0.25 with resin quantity, canal density and percentage canal area respectively), while the modulus of elasticity had a moderate and negative correlation to resin features (r = -0.23, -0.29 and -0.32 with resin quantity, canal density and percentage canal area respectively).

Limiting factors for this study include:

- The resin quantity was estimated by NIR on solid wood surface. The relative values were not validated by actual resin quantity which can be measured by extracting milled wood with an organic solvent like acetone. Resin extraction of milled wood with a solvent would account for variation in resin quantity on the surface of the samples and verify the spectroscopic assessment.
- The 2 year-old wood materials were free of decay when used in this study but they were stored for over two years. It is possible that small amounts of resin were lost during storage. If this were the case it could have reduced the resin quantity values estimated for each sample.
- Because of time and resource constraints the preparation of wood sections was limited to 10 samples for each family and clone (sample size = 300). NIR spectroscopy considered all samples available for each material type (sample size = 600).

Future work should include:

- Some samples should be milled for chemical extraction of resin to validate the NIR method used in this study.

- The study should be replicated at other sites across New Zealand to establish the influence of the environment on resin features among the radiata pine genetic materials available in New Zealand.
- The study should be replicated with older radiata pine of the same genotypes. This will determine how the parameters are influenced by tree age.
- Not all families were included into the study and for this reason families not covered by this study need to be assessed in future if selection for resin features is desired.
- Variation in actual chemical components of resin from the genetic materials could be explored.

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Appendices

Appendix A: Summary statistics of resin amount for a 2-year old wood from radiata pine clones and families. c = clones, f = families.

	Amount of resin			
Planting material	Mean (au)	CV (%)	Min	Max
c-A	2.18	0.45	0.31	3.66
c-B	1.85	0.56	0.61	0.61
c-C	3.47	0.47	0.47	0.80
c-D	2.62	0.66	0.66	0.72
c-E	2.87	0.73	0.74	0.15
c-F	3.39	0.48	0.48	0.92
c-G	3.17	0.54	0.54	0.55
c-H	2.70	0.69	0.69	0.45
c-I	2.64	0.66	0.66	0.48
c-J	2.32	0.61	0.61	0.52
f-A	3.10	0.50	0.50	0.83
f-B	4.72	0.55	0.55	1.51
f-C	4.23	0.52	0.52	1.43
f-D	3.45	0.51	0.51	1.04
f-E	3.25	0.55	0.55	0.38
f-F	3.84	0.58	0.58	0.65
f-G	3.27	0.61	0.61	0.70
f-H	3.93	0.40	0.40	1.26
f-I	4.36	0.50	0.50	1.06
f-J	3.54	0.44	0.44	1.21

f-K	3.46	0.41	0.41	1.11
f-L	3.48	0.62	0.62	0.83
f-M	3.95	0.41	0.41	1.04
f-N	3.86	0.38	0.38	1.60
f-O	4.20	0.51	0.51	1.07
f-P	3.15	0.40	0.40	1.67
f-Q	3.82	0.52	0.52	1.32
f-R	3.72	0.40	0.40	1.56
f-S	3.63	0.47	0.47	0.70
f-T	2.40	0.55	0.54	0.80

Appendix B: Summary statistics for resin canal density for 2-year old wood from radiata pine clones and families. c=clones, f=families.

	Resin canal density			
Planting material	Mean (canal/mm^2)	CV (%)	Min	Max
c-A	0.83	0.33	0.52	1.42
c-B	0.45	0.33	0.28	0.77
c-C	0.67	0.25	0.47	1.01
c-D	0.64	0.23	0.48	0.89
c-E	0.62	0.19	0.47	0.84
c-F	0.57	0.27	0.31	0.78
c-G	0.77	0.26	0.50	1.18
c-H	0.75	0.24	0.47	1.00

c-I	0.65	0.37	0.37	1.09
c-J	0.90	0.24	0.61	1.21
f-A	1.00	0.30	0.50	1.36
f-B	1.00	0.26	0.62	1.31
f-C	0.81	0.22	0.59	1.17
f-D	0.80	0.30	0.39	1.32
f-E	0.74	0.41	0.37	1.33
f-F	0.88	0.26	0.55	1.32
f-G	0.91	0.21	0.69	1.35
f-H	0.96	0.27	0.60	1.34
f-I	1.11	0.32	0.67	1.91
f-J	0.80	0.22	0.52	1.04
f-K	0.84	0.28	0.64	1.36
f-L	0.79	0.26	0.54	1.27
f-M	0.92	0.17	0.69	1.18
f-N	0.95	0.22	0.49	1.21
f-O	0.90	0.25	0.62	1.37
f-P	0.94	0.23	0.6	1.33
f-Q	1.12	0.36	0.73	2.00
f-R	0.77	0.13	0.55	0.87
f-S	1.00	0.17	0.77	1.23
f-T	0.86	0.22	0.64	1.29

Appendix C: Summary statistics for % resin canal area for 2-year old wood from radiata pine clones and families. c = clones, f = families.

% resin canal area				
Planting material	Mean	CV (%)	Min	Max
c-A	1.39	0.29	0.88	2.27
c-B	0.90	0.33	0.52	1.36
c-C	1.14	0.23	0.81	1.72
c-D	1.05	0.24	0.76	1.44
c-E	1.06	0.21	0.75	1.43
c-F	0.93	0.25	0.44	1.26
c-G	1.19	0.31	0.79	1.94
c-H	1.27	0.25	0.75	1.69
c-I	1.00	0.43	0.60	1.85
c-J	1.38	0.23	0.93	1.93
f-A	1.75	0.31	1.01	2.66
f-B	1.62	0.27	1.10	2.36
f-C	1.47	0.25	1.01	2.11
f-D	1.22	0.37	0.78	2.37
f-E	1.25	0.44	0.56	2.26
f-F	1.50	0.24	1.07	2.24
f-G	1.59	0.28	1.03	2.70
f-H	1.70	0.28	1.08	2.48
f-I	1.91	0.36	1.10	3.43

f-J	1.38	0.20	0.88	1.87
f-K	1.52	0.23	1.16	2.18
f-L	1.39	0.26	0.98	2.29
f-M	1.51	0.14	1.19	1.77
f-N	1.62	0.20	0.88	1.94
f-O	1.55	0.26	0.99	2.46
f-P	1.45	0.21	0.90	1.88
f-Q	1.88	0.28	1.24	2.98
f-R	1.29	0.15	0.88	1.56
f-S	1.65	0.17	1.30	2.02
f-T	1.39	0.23	0.94	2.07

Appendix D: Summary statistics for resin canal size for 2-year old wood from radiata pine clones and families. c = clones, f = families.

	Resin canal size			
Planting material	Mean (mm^2)	CV (%)	Min	Max
c-A	0.0170	0.14	0.014	0.021
c-B	0.0181	0.11	0.040	0.020
c-C	0.0172	0.14	0.015	0.023
c-D	0.0164	0.09	0.015	0.020
c-E	0.0171	0.09	0.015	0.019
c-F	0.0164	0.11	0.014	0.020
c-G	0.0155	0.14	0.013	0.021

c-H	0.0170	0.09	0.015	0.019
c-I	0.0151	0.09	0.013	0.017
c-J	0.0154	0.07	0.014	0.017
f-A	0.0175	0.12	0.015	0.020
f-B	0.0169	0.06	0.015	0.018
f-C	0.0181	0.08	0.016	0.020
f-D	0.0153	0.15	0.013	0.020
f-E	0.0166	0.10	0.015	0.019
f-F	0.0173	0.11	0.015	0.021
f-G	0.0174	0.11	0.015	0.021
f-H	0.0178	0.07	0.015	0.019
f-I	0.0172	0.15	0.015	0.023
f-J	0.0175	0.09	0.015	0.020
f-K	0.0184	0.10	0.016	0.022
f-L	0.0177	0.08	0.015	0.019
f-M	0.0166	0.09	0.015	0.019
f-N	0.0171	0.06	0.015	0.018
f-O	0.0173	0.08	0.015	0.019
f-P	0.0155	0.05	0.014	0.017
f-Q	0.0172	0.13	0.014	0.021
f-R	0.0168	0.06	0.015	0.019
f-S	0.0165	0.07	0.014	0.018
f-T	0.0162	0.10	0.014	0.020

Appendix E: Testing for significant differences in means for variables with ANOVA across clones

Summary of ANOVA for resin content in radiata pine clones

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Clones	9	0.0000466	5.178e-06	1.964	0.0458 *
Residuals	181	0.0004771	2.636e-06		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Summary of ANOVA for % resin canal area in radiata pine clones

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Clones	9	2.727	0.303	2.97	0.00387 **
Residuals	90	9.181	0.102		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Summary of ANOVA for resin canal density in radiata pine clones

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Clones	9	1.333	0.14814	4.016	0.000241 ***
Residuals	90	3.320	0.03689		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Summary of ANOVA for canal size in radiata pine clones

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genetic	9	8.096e-05	8.996e-06	2.681	0.00832 **
Residuals	90	3.020e-04	3.356e-06		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix F: Testing for significant differences in means for variables with ANOVA across families

Summary of ANOVA for resin quantity in radiata pine families

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	19	0.0001017	5.352e-06	1.58	0.0578 .
Residuals	389	0.0013174	3.387e-06		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Summary of ANOVA for % resin canal area in radiata pine families

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	19	6.868	0.3615	2.135	0.0055 **
Residuals	180	30.479	0.1693		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Summary of ANOVA for canal density in radiata pine families

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	19	2.159	0.11364	1.971	0.0119 *
Residuals	180	10.380	0.05767		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Summary of ANOVA for canal size in radiata pine families

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	19	0.0001159	6.100e-06	2.294	0.00255 **
Residuals	180	0.0004787	2.659e-06		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1