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The potential for using foliar carbon isotopic signature to screen drought tolerant radiata pine genotypes for dryland plantation forests in New Zealand

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The potential for using foliar carbon isotopic signature to screen drought tolerant radiata pine genotypes for dryland plantation forests in New Zealand

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REPORT INFORMATION SHEET

REPORT TITLE	THE POTENTIAL FOR USING FOLIAR CARBON ISOTOPIC SIGNATURE TO SCREEN DROUGHT TOLERANT RADIATA PINE GENOTYPES FOR DRYLAND PLANTATION FORESTS IN NEW ZEALAND
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EXECUTIVE SUMMARY

Objective

The aim of this study was to assess the potential use of $\delta^{13}C$ in a future breeding program for selection of radiata pine genotypes with high water use efficiency (WUE) and better growth performance under drought-prone conditions through quantifying the genetic variation in needle $\delta^{13}C$ (a surrogate index of WUE), height and diameter growth of 20 individual trees of 120 open pollinated radiata pine families, and determining the genetic correlations among these traits.

Key Results

1. At age 7.5 years, the large phenotypic variation was found for needle $\delta^{13}C$, which varied over a range of -5.09‰ (28.34‰ ~ 23.25‰, equivalent to WUE_i of 60-113 μmol CO_2 mol^{-1} H_2O) among individual trees.

2. The five traits measured at age 7.5 years showed variable levels of genetic control. The narrow-sense heritability estimates were very low for stem malformation (0.09 ± 0.03), low for stem straightness (0.16 ± 0.05), tree height (0.12 ± 0.03) and DBH (0.20 ± 0.04), but moderate for needle $\delta^{13}C$ (0.40 ± 0.08).

3. At age 7.5 years, the phenotypic correlations were low between needle $\delta^{13}C$ and tree height (r = 0.09 ± 0.02), and DBH (r = 0.18 ± 0.02), but strong between tree height and DBH (r = 0.50 ± 0.02).

4. A moderate positive genetic correlation was observed between needle $\delta^{13}C$ and tree DBH (r = 0.43 ± 0.13), but a low positive one between needle $\delta^{13}C$ and tree height (r = 0.22 ± 0.17) at age 7.5 years.

Implications of Results/Conclusions

This study demonstrated significant genetic variation and moderate heritability for needle $\delta^{13}C$, indicating that this trait was heritable for radiata pine in New Zealand. The moderate and positive genetic correlations between the needle $\delta^{13}C$ and growth traits suggest the variation of $\delta^{13}C$ among individual trees in this breeding population was mainly controlled by photosynthetic capacity. Our results highlight the potential use of needle $\delta^{13}C$ as a useful trait for indirectly selecting radiata pine genotypes with improved WUE and better growth performance under dry conditions.

Further Work

In view of the questions raised by some of the data presented in this report, the following is recommended:

1. Genetic expression of needle $\delta^{13}C$ may vary with the environment, and therefore the genetic parameters need to be assessed on contrasting sites with different soil moisture stress to explore the importance of genotype x environment interaction.

2. It is important to determine the genetic variation and parameters for needle $\delta^{13}C$ and growth traits within and between populations and families of radiata pine to develop forward or backward selection strategies.

3. Application of the $\delta^{13}C$ technique to select water-use efficient genotypes in the deployment population is recommended. We recommend measuring $\delta^{13}C$ throughout commercial orchards and /or the RPBC production population to select already productive genetic material for drought tolerant parents that can be rapidly deployed in drier climates.

4. In the longer term we recommend that elite germplasm and/or production population candidates are tested on drier sites to ensure future production population parents can be screened for improved WUE and drought tolerance so that New Zealand is prepared for planting a larger number of drought-prone sites.

The potential for using foliar carbon isotopic signature to screen drought tolerant radiata pine genotypes for dryland plantation forests in New Zealand

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June 2012

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Introduction

Drought and drought tolerance

With climate warming, large areas of New Zealand are expected to become drier and more prone to drought. NIWA predicts that under the 'medium-high' scenario, severe droughts are projected to occur more than four times more frequently by the 2080s in the eastern parts of North Otago, Canterbury and Marlborough, Wairarapa, Bay of Plenty and Coromandel, Gisborne and Northland (Mullan et al., 2005). Increased frequency and severity of droughts caused by climate change is a major risk to the productivity and health of New Zealand's planted forests, which depend on moisture to maintain high levels of carbon sequestration and storage. This could have a serious impact on export earnings and the communities associated with our forests. Drought causes high tree mortality and can reduce radiata pine (*Pinus radiata*) productivity by up to 16 m^3/ha/year (Watt et al., 2010). When applied to east coast forests alone this could equate to a loss of $38M per annum. Total costs to New Zealand from the 1997/98 drought are estimated at $1 billion (http://www.maf.govt.nz/climatechange/about/1-3-new-zealand-perspective.htm). Increasing drought costs are expected with rising temperatures and decreased rainfall under future climates.

With these risks identified, the New Zealand forestry industry needs to develop strategies to either mitigate or adapt to these impacts. There are two principle sets of management tools that foresters can use to reduce the impact of drought stress on forest survival and growth; the identification and deployment of drought tolerant species and genotypes and the use of silvicultural options such as irrigation, weed control and mulching, etc. However, silvicultural treatments may not be affordable or practical in many instances, especially on a large scale. The selection for drought tolerant species and genotypes offers the first major impact point across the forestry value chain. This topic is a key area of forest research worldwide (e.g. Picon et al., 1996; Pita et al., 2001, 2005; Guehl, 2002; Sonesson and Eriksson, 2003; Cregg, 2004; Sofo et al., 2008; Roussel et al., 2009a). The development of drought tolerant and water efficient forest species and genotypes has been recognised internationally (e.g. Australian Low Rainfall Tree Improvement Group, Boardman et al., 2002;) as a potentially very important and economically attractive means to adapt forest production to climate change, and to address current challenges of water crisis faced by many countries. Planting forest species and genotypes adapted to a future warmer and drier climate will not only improve forest productivity in drought prone environments, but could also contribute to the mitigation of climate change impact through increased efficiency of water use and carbon sequestration.

Drought tolerance refers to the degree to which a plant is adapted to arid or drought conditions. Plants use various mechanisms to cope with drought stress. These can broadly be classified into two groups; dehydration avoidance and dehydration tolerance (Levitt, 1980; Ludlow, 1989). Dehydration avoidance involves strategies which help the plant maintain an adequate water status during periods of stress, either by efficient water absorption from roots or by reducing evapo-transpiration from aerial part (Ludlow, 1989; Lopes and Reynolds, 2010), and mechanisms related to increased water use efficiency (Araus et al., 2002; Condon et al., 2002). Dehydration tolerance, which is found in species with lower stomatal sensitivity but displaying structural and functional adaptive traits such as osmoregulation, allows the plant to maintain turgor and continue metabolism even at low water potential, e.g. by protoplasmic tolerance, synthesis of osmoprotectants, osmolytes or compatible

solutes (Levitt, 1980; Ludlow, 1989). The range of tolerance to dehydration would depend on the species and stage of development.

Drought tolerance is a complex biological trait with diverse drought-adaptive structural and functional mechanisms involved in using water efficiently (Ludlow, 1989; Blum, 2005; Reynolds and Tuberosa, 2008; Sofo et al., 2008; Pinto et al., 2010). One approach being used is to identify some morpho-physiological traits involved in improving water use efficiency to speed up the breeding of drought tolerant crop genotypes (e.g. Cregg and Zhang, 2000; Condon et al., 2002, 2004; Cregg, 2004; Saint Pierre et al., 2012).

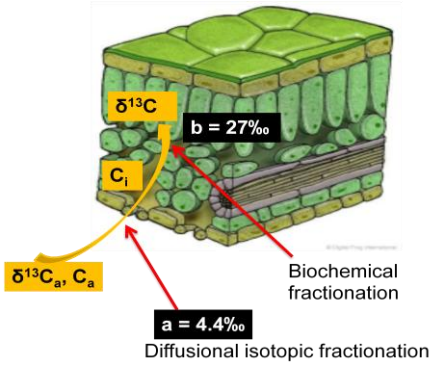
Water use efficiency and carbon isotope discrimination

Water use efficiency (WUE) is a composite and complex trait that currently receives much attention from agronomists, ecophysiologists, and geneticists (Condon et al., 2004). A goal of modern agriculture and forestry is to improve plant drought tolerance and production per amount of water used, referred to as WUE. WUE can be considered at many levels, from field to plant to single leaf scale. At the leaf scale WUE can be defined as the ratio between rate of CO_2 assimilation (A) and rate of transpiration (E), i.e. instantaneous water use efficiency. Because E is highly dependent on temperature and relative humidity (encapsulated in the term of vapour pressure deficit (VPD) for H_2O between leaf and atmosphere), WUE is often defined as the ratio between A and stomatal conductance (g_s), i.e. intrinsic WUE (WUE_i) - a measure that is independent of VPD.

Two fundamental processes that influence biomass accumulation and water loss occur primarily in leaves; (i) net diffusion of CO_2 into the leaf through stomata and into sub-stomatal airspaces followed by assimilation of CO_2 by photosynthesis, largely in the palisade cells, and (ii) transpiration whereby evaporation occurring mostly at the cell surfaces in the sub-stomatal spaces is followed by a net diffusion of water vapour out of the leaf through the stomatal pores.

In leaves, instantaneous A/E and A/g_s ratios can be measured using infrared gas-exchange equipment, and surrogate measures of A/g_s that are time-integrated over leaf development can be obtained by measuring stable carbon isotope discrimination ($\Delta^{13}C$), a relatively high-throughput method.

The isotopic ratio of ^{13}C to ^{12}C in plant tissue is less than the isotopic ratio of ^{13}C to ^{12}C in the atmosphere, indicating that plants discriminate against ^{13}C during photosynthesis. The isotopic ratio of ^{13}C to ^{12}C in C_3 plants varies mainly due to discrimination during diffusion and enzymatic processes.



The rate of diffusion of $^{13}CO_2$ across the stomatal pore is lower than that of $^{12}CO_2$ by a factor of 4.4‰. Additionally, there is an isotope effect caused by the preference of ribulose bisphosphate carboxylase (*Rubisco*) for $^{12}CO_2$ over $^{13}CO_2$ (by a factor of ~27‰). *Rubisco* is an enzyme involved in the first major step of carbon fixation, a process by which atmospheric carbon dioxide is converted by plants to energy-rich molecules such as glucose.

In both cases, the processes discriminate against the heavier isotope, ^{13}C (Farquhar et al., 1989a). Based on the work of Farquhar and his colleagues the linkage between discrimination against ^{13}C during photosynthesis and water use efficiency can be demonstrated by the following relationships. The carbon isotope composition ($\delta^{13}C$) is expressed as the $^{13}C/^{12}C$ ratio relative to the Pee Dee Belemnite standard as (Craig, 1957):

$$\delta^{13}C\ (‰) = ((R_{sa}/R_{st}) - 1)\ 1000 \qquad [1]$$

where R_{sa} and R_{st} are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively. The discrimination (Δ) between $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C_{air} \approx -8‰$) and $\delta^{13}C$ of plant material ($\delta^{13}C_{plant}$) is calculated as (Farquhar and Richards, 1984):

$$\Delta = (\delta^{13}C_{air} - \delta^{13}C_{plant})/(1+ \delta^{13}C_{plant}) \qquad [2]$$

Intrinsic WUE (WUE_i) can be estimated from discrimination (Δ) using a modified equation (Brendel et al., 2002) from Farquhar et al. (1982):

$$WUE_i\ (\text{mol } CO_2/\text{mol } H_2O) = A/g_s = C_a/1.6 \times ((b-\Delta)/(b-a)) \qquad [3]$$

where C_a is the atmospheric CO_2 concentration ($\approx 360 \times 10^{-6}$ mol mol^{-1}), a (4.4‰) is discrimination occurring during diffusion of CO_2 in air, b (27‰) is discrimination caused by carboxylation and Δ is the discrimination between $\delta^{13}C_{air}$ and $\delta^{13}C_{plant}$ (Equation 2).

Based on the relationship described above (Equation 3), Δ is linked to WUE_i through the effects of A and g_s on intercellular CO_2. As WUE_i increases due to stomatal closure (decrease g_s) or an increase in A, intercellular CO_2 declines and discrimination decreases. Therefore, WUE_i is negatively related to Δ and positively related to $\delta^{13}C$.

A strong correlation between WUE_i and Δ or $\delta^{13}C$ has been reported for numerous crop and tree species (e.g. Sun et al., 1996; Condon et al., 2004; Cregg, 2004; Roussel et al., 2009b). For example, Johnson et al. (1993) reported that correlations between Δ and A/g_s ranged between –0.77 and –0.91 for crested wheatgrass in a series of greenhouse and field studies. In the same trials the correlation between Δ and transpiration efficiency ranged between –0.73 and –0.94. In a study of western larch (*Larix occidentalis* Nutt.) seedlings, Zhang and Marshall (1994) found that Δ was significantly correlated with transpiration efficiency (r= –0.85) and instantaneous water use efficiency (r = –0.70). Roussel et al. (2009b) reported that correlations between $\Delta^{13}C$ and WUE_i were strong (r = –0.70 to –0.94) among *Quercus robur* genotypes. It has also been reported that seasonal variation in WUE_i or water stress can be assessed by intra-ring $\delta^{13}C$ for *Pinus radiata* (Walcrofts et al., 1997; Barbour et al., 2002), *Pinus pinaster* (Nguyen-Queyrens et al., 1998; Porté and Loustau, 2001) and *Quercus petraea* species (Michelot et al., 2011).

Carbon isotope discrimination (Δ) or composition ($\delta^{13}C$) has several conceptual and logistical advantages to screening for drought tolerance based on A/E or A/g_s (i.e. WUE_i). Δ or $\delta^{13}C$ is attractive because it provides a time and spatially integrated measure of the balance among the important traits influencing carbon gain and water use by plants. Δ or $\delta^{13}C$ has been shown to have substantial potential application as a screening tool in breeding programs to select genotypes with greater WUE_i and productivity under drought conditions.

Genetic variation in Δ (or $\delta^{13}C$) in relation to tree growth

Genetic variation in foliar or tree ring carbon isotope discrimination (Δ) or composition ($\delta^{13}C$) has been reported in tree species, provenances, populations, family and clones. For example, significant intra-specific variation in Δ or $\delta^{13}C$ has been observed in several conifers, including *Pseudotsuga menziesii* (Mirb.) Franco (Zhang et al., 1993; Zhang and Marshall, 1995; Aitken et al., 1995), *Picea* species and hybrids (Flanagan and Johnsen, 1995; Sun et al., 1996; Johnsen et al., 1999; Silim et al., 2001), *Larix occidentalis* Nutt. (Zhang and Marshall, 1994; Zhang et al., 1994), and *Pinus* species and hybrids (Zhang and Cregg, 1996; Zhang et al., 1997; Nguyen-Queyrens et al., 1998; Olivas-Garcia et al., 2000; Brendel et al., 2002; Prasolova et al., 2003; Emhart, 2005; Baltunis et al., 2008; Xue et al., 2009). Genetic variation in Δ or $\delta^{13}C$ has not been well explored for *Pinus radiata*, a significant softwood plantation species worldwide, and the most common softwood species under plantation in temperate zones of the southern hemisphere (Rowell et al., 2009). No significant genetic variation in cellulose $\delta^{13}C$ of tree rings has been reported for both 7 OP families and 8 full-sib families of *Pinus radiata* (Rowell et al., 2009). However, significant genetic variation in needle $\delta^{13}C$ has been observed for 40 *Pinus radiata* clones, with clonal repeatability of 0.46 (Xue et al., 2012).

The genetic variation in Δ or $\delta^{13}C$ has been found to be associated with the differences in stomatal conductance (Bond and Stock, 1990; Prasolova et al., 2000; Correia et al., 2008), or photosynthetic capacity (Zhang et al., 1993; Flanagan and Johnsen, 1995; Johnsen et al., 1999; Prasolova et al., 2001, 2003; Xu et al., 2003; Rasheed et al., 2012), or both (Pennington, et al., 1999; Monclus et al., 2005; Bonhomme et al., 2008). It has been suggested that provenance differences of $\delta^{13}C$ in conifer species might be determined by differences in stomatal sensitivity to changes in vapour pressure deficit (Zhang and Marshall, 1995) and/or differences in plant hydraulic characteristics (Guehl et al., 1995). However, differences of $\delta^{13}C$ among families within provenances of *Picea mariana* (Mill.) Britton have been found to be mainly determined by differences in photosynthetic capacity (Johnsen and Major, 1995; Major and Johnsen, 1996; Johnsen et al., 1999). Similar results have been reported for families of *Pinus pinaster* (Ait.) (Guehl et al., 1995), families of *Araucaria cunninghamii* Ait. ex D. Don (Prasolova et al., 2001; Xu et al., 2003), and the clones of the F1 hybrid between *Pinus elliottii* Engelm x *Pinus caribaea* Morelet growing in a drought-prone environment (Xu et al., 2000; Prasolova et al., 2003).

Tree growth is an important goal for forest tree breeding programmes and is one of the key traits for the Radiata Pine Breeding Company (e.g. DBH). To avoid inadvertent negative selection for growth when selecting for high WUE_i, it is important to know if tree growth and isotope carbon composition ($\delta^{13}C$) or discrimination (Δ) are genetically correlated. $\delta^{13}C$ values should be positively (or Δ negatively) related to productivity when variation in discrimination is a result of changes in carboxylation efficiency (i.e. photosynthetic capacity). In contrast, if variation in discrimination is related primarily to variation in stomatal conductance, then $\delta^{13}C$ values should be negatively (or Δ positively) correlated with growth. Strong and positive phenotypic correlations have been reported between leaf $\delta^{13}C$ and tree diameter ($r = 0.92$, $P = 0.03$) for *Larix occidentalis* Nutt. (Zhang et al., 1994), between tree ring $\delta^{13}C$ and height ($r = 0.80$, $P = 0.01$) and between tree ring $\delta^{13}C$ and width ($r = 0.46$, $P = 0.005$) for *Pinus pinaster* (Nguyen-Queyrens et al., 1998; Brendel et al., 2002). Significant and positive genetic correlations have been reported between leaf $\delta^{13}C$ and tree height ($r = 0.98$) or diameter ($r = 0.64$) for *Picea mariana* Mill. (Johnsen et al., 1999), between leaf $\delta^{13}C$ and tree height ($r = 0.96$) for hybrid pine clones of *Pinus elliottii* Engelm x *Pinus caribaea* Morelet (Xu et al., 2000), between leaf $\delta^{13}C$ and tree DBH

(r = 0.38) for families of *Araucaria cunninghamii* (Xu et al., 2003), and between leaf $\delta^{13}C$ and tree height (r = 0.54) for families of *Pinus taeda* (Baltunis et al., 2008). The genetic correlations between $\delta^{13}C$ and various above- and below-ground growth traits in Castanea sativa Mill. were generally strong and positive (Lauteri et al., 2004). In contrast, the negative phenotypic correlations have been observed for *Eucalyptus globulus* Labill. (Bond and Stock, 1990, Osorio and Pereira, 1994, Pita et al., 2001), *Fagus sylvatica* L. (Dupouey et al., 1993) and *Pinus strobus* (McNulty and Swank, 1995). No significant phenotypic or genetic correlations between $\delta^{13}C$ and tree growth have also been reported for *Populus* × *euramericana* (Monclus et al., 2005, 2006; Bonhomme et al., 2008; Rasheed et al., 2011) and for *Pinus pinaster* (Brendel et al., 2002). Johnsen et al. (1999) found strong and positive genetic correlations between $\delta^{13}C$ and tree growth (height and DBH) of *Picea mariana* Mill. They concluded that photosynthetic capacity (*A*) was determining $\delta^{13}C$ and growth performance and thus constituted probably the link between $\delta^{13}C$ and tree growth. However, since $\delta^{13}C$ (or Δ) as an indicator of WUE_i can be either controlled by *A* and/or by stomatal conductance (g_s), there is not necessarily a strong relationship between growth and $\delta^{13}C$ (or Δ). This suggests the existence of a genetic correlation between $\delta^{13}C$ (or Δ) and growth is dependent on the factor by which WUE_i is controlled, but the size, direction and significance of the correlation appears to be species dependent.

Although selection of high WUE_i based on $\delta^{13}C$ (or Δ) for better growth and yields under drought conditions has been reported in several agricultural and forest crops worldwide (e.g. Cregg and Zhang, 2000; Rebetzke et al., 2002; Condon et al., 2004; Gebrekirstos et al., 2011; Sinclair, 2012), very few studies have reported on the use of $\delta^{13}C$ (or Δ) in tree improvement programs of radiata pine (Rowell et al., 2009), especially in New Zealand, where radiata pine is the key plantation forest species. The use of $\delta^{13}C$ (or Δ) signature offers the potential to select radiata pine genotypes that will maintain or improve productivity under drought stress. The potential of using $\delta^{13}C$ as a screening indicator for WUE has been tested for radiata pine in previous studies (Xue et al., 2004, 2009, 2011, 2012). However, these trials were not designed to estimate heritability and the genetic correlations between $\delta^{13}C$ and growth traits, and to rigorously evaluate its potential use for screening drought tolerant radiata pine genotypes in a breeding framework. The present study will determine whether the $\delta^{13}C$ technique will be applicable for screening the breeding population by testing its application at one breeding trial at a dry site.

The objectives of this study are: (1) to quantify the genetic variation in needle $\delta^{13}C$, tree growth and form among individual trees of 120 open pollinated radiata pine families, and the genetic correlations among these traits; (2) to determine if genetic variation in needle $\delta^{13}C$ is associated with the differences in stomatal conductance or photosynthetic capacity by examining the genetic correlation between needle $\delta^{13}C$ and tree growth; (3) to assess the potential use of $\delta^{13}C$ in a future breeding program for selection of radiata pine genotypes with high WUE for better growth performance under drought-prone conditions.

Materials and Methods

Site descriptions and soil characteristics

The RPBC (Radiata Pine Breeding Company) breeding trial assessed in this study is located at Rolleston, Christchurch, New Zealand (43°37'S, 172°21'E). The site is flat

and at an altitude of 45 m. The current rotation is the first for radiata pine on the ex-pasture site. This site has low rainfall, high wind speed (Table 1) and summer (Dec.-Feb.) temperature (mean midday temperature 23 ^{o}C). The soil at the site is Lismore stony silt loam (Table 1) with a low water holding capacity, natural fertility and considered marginal for agriculture and forestry (New Zealand Soil Bureau 1968). The parent material is aggradation gravel with partial glacial gravel.

Table 1: Climatic conditions and selected soil properties* (0-10 cm depth) for the breeding trial at a dry site near Christchurch.

Parameter	
[a] Mean annual rainfall (mm)	648
[a] Mean annual temperature (^{o}C)	11.5
[a] Mean annual sunshine hours	2100
[a] Mean wind speed (km hr^{-1})	15
[b] Total rainfall during growing season (Sept. – Feb.) (mm)	223
[c] Mean temperature (Sept. – Feb.) (^{o}C)	13.7
Soil type	Lismore stony silt loam
NZ classification (Hewitt 98)	Pallic Firm Brown Soils
US Taxonomy	Udic Haplustepts
pH	4.8
Total N (g 100g^{-1})	0.24
Total C (g 100g^{-1})	3.1
Total C/N	13
Total P (g 100g^{-1})	0.034
Olsen P (mg kg^{-1})	11.4
Exchangeable K (cmol$_c$ kg^{-1})	0.29
Exchangeable Na (cmol$_c$ kg^{-1})	0.09
Exchangeable Ca (cmol$_c$ kg^{-1})	2.24
Exchangeable Mg (cmol$_c$ kg^{-1})	0.92
Cation exchange capacity (cmol$_c$ kg^{-1})	0.33

* The soil properties were measured at another trial next to this RPBC breeding trial.
[a] All the climatic data are the mean values for the period of 1971-2000. [b] Total rainfall during growing season (Sept – Feb.) is the cumulative values of the mean monthly rainfall from Sept. to Feb., which are averaged across 30 years (1971-2000). [c] Mean temperatures are the mean values of the mean monthly temperature from Sept. to Feb., which are averaged across 30 years (1971-2000).

Genetic material and trial design

The trial used in this study is one of the nationwide breeding trial series established by the Radiata Pine Breeding Company (RPBC) (www.rpbc.co.nz). The trial was established at Rolleston, Christchurch, with 122 open-pollinated families (OP) from the New Zealand radiata pine breeding programme (Dungey et al., 2009). Eight control families or seedlots (99/188, 97/067, 99/378, 99/318, 97/062, GF7, GF14, and GF19) were also included.

The trial is a sets-in-replicates single-tree-plot design, with 30 replications at the site (Appendix 1). This has long been the standard field layout for progeny trials in New Zealand because of its combination of good precision, logistical convenience, and ease of data analysis. The sets (A, B, C and D) were used to simplify the trial preparation. The equal numbers of different families were randomly allocated to each

set (i.e. A, B, C or D) within replicate to minimise the likelihood of a set effect. The trial was established in August 2004 at 4 x 4 metre spacing.

Trial measurements

The trial was measured for different traits for 122 OP families at 20 randomly selected replications (excluding 2, 7, 15, 21-27 (A, B, C, D) replicates) in February 2012 (7.5 years old). The traits measured were:

- Current-year full mature needles for carbon isotope composition ($\delta^{13}C$).
- Tree height and diameter at breast height (DBH).
- Stem straightness and malformation.

The carbon isotope ratio ($^{13}C/^{12}C$) was measured in the needle samples collected from all individual trees in the 4 sets (i.e. plots) within each of 20 selected replications in mid February 2012. For each individual tree (i.e. each OP family), three branchlets with full mature current-year needles were collected from the north-facing (sun-exposed) youngest second-order branches of top third crown. All mature current-year needles from three branchlets of the same individual tree were bulked together as one sample per tree. The 2400 needle samples (120 OP families x 20 replications) were dried at 65°C and ground to a fine powder (< 0.14-mm) with a mortar and pestle or with a tissue grinder. The needle $^{13}C/^{12}C$ ratio was determined on all samples by the Stable Isotopes Unit at Waikato University using continuous-flow isotope ratio mass spectrometry (Europa Scientific Ltd., Crewe, UK). The carbon isotope composition ($\delta^{13}C$) was estimated as the sample $^{13}C/^{12}C$ ratio relative to the Pee Dee Belemnite standard as (Craig 1957):

$$\delta^{13}C\ (‰) = ((R_{sa}/R_{st}) - 1)\ 1000$$

where R_{sa} and R_{st} are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively.

Tree height (m) and diameter at breast height (DBH, mm) at 1.4 m above ground were measured for all individual trees at sets A, B, C and D (i.e. plots) within each of 20 selected replications in late February 2012 soon after collection of needle samples for $\delta^{13}C$ analysis.

Based on the description of NZRPBC (1997), stem straightness (Figure 1) was assessed on a scale of 1 (worst) to 9 (best) and stem malformation (Figures 2) on a scale of 1 (multi-leaders) to 9 (single dominant leader) for all individual trees at A, B, C and D sets (i.e. plots) within each of 20 selected replications in late February 2012. This methodology is used for assessing RPBC trials and is therefore compatible with standard radiata pine breeding practice.

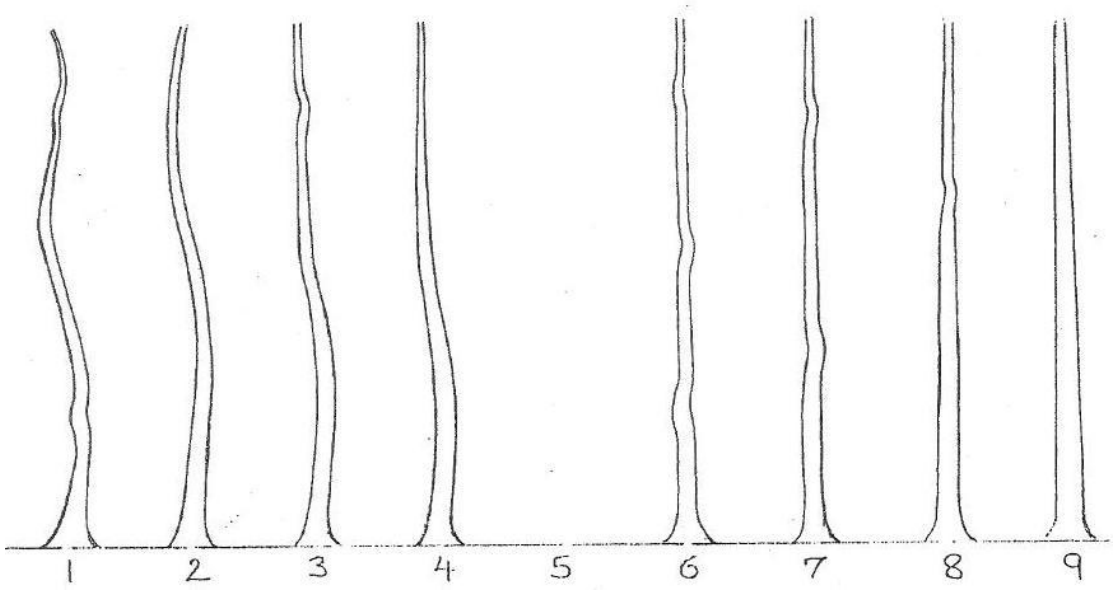


Figure 1. Stem straightness score for radiata pine stem quality. Class 5 is not scored to avoid central bias.

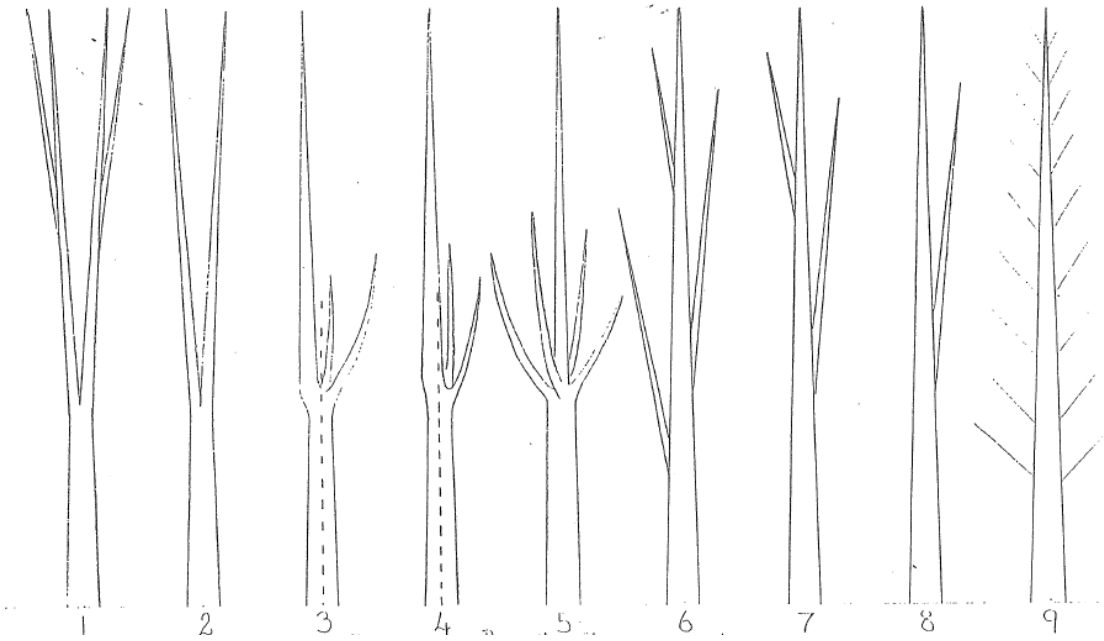


Figure 2. Stem malformation score for radiata pine stem quality.

Statistical analysis

Summary statistics were obtained using R (R Development Core Team, 2012) and genetic analysis was undertaken using ASReml (Gilmour et al., 2010).

Data analysis was undertaken according to the following general linear mixed model:

$$\mathbf{y = Xb + Zu + e} \quad [4]$$

where **y** is a vector of individual tree observations for a trait, **b** is a vector of fixed effects, **u** is a vector of random effects, **e** is a vector of random residuals, and **X** and

Z correspond to design matrices relating the observations in **y** to the fixed and random effects in **b** and **u**, respectively. The joint distribution of the random terms was assumed to be multivariate normal, with means and (co)variances defined as:

$$\begin{bmatrix} \mathbf{u} \\ \mathbf{e} \end{bmatrix} \sim N\left(\begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{G} & \mathbf{0} \\ \mathbf{0} & \mathbf{R} \end{bmatrix} \right) \quad [5]$$

where **0** is a null matrix, and **G** and **R** are (co)variance matrices for effects in **u** and **e**, respectively. Restricted maximum likelihood (REML) estimates of (co)variance parameters and their standard errors were obtained by using the average information REML algorithm implemented in the ASREML program (Butler et al., 2009; Gilmour et al., 2010). The analyses assumed that epigenetic effects (Burdon and Shelbourne, 1974) were negligible for all traits in the studied population. The following analyses were undertaken.

Fixed terms in vector **b** included a factor with two levels to account for the effects of controls versus genetic (open-pollinated family) material. Random terms in vector **u** included the additive genetic effects of individual genotypes within the genetic material, and factors to account for Replicate, and Replicate x Sets interaction. All the effects in **u** were assumed to be mutually independent, and thus **G** was defined as a direct sum of submatrices $\mathbf{G}_i$, where $\mathbf{G}_i$ is the (co)variance matrix for the i^{th} random term.

To estimate additive genetic correlations, the $\mathbf{G}_i$ for the additive genetic effects was defined as **C** ⊗ **A,** where **C** is a 2 x 2 matrix with additive genetic variances for two different traits (e.g. DBH, height, $\delta^{13}C$) as the diagonal elements and the additive genetic covariance between the two traits on the off-diagonal, **A** is the numerator relationship matrix (Henderson, 1984), and ⊗ is the Kronecker product. Phenotypic correlations between trait 1 and trait 2 were estimated using the sum of the variance/covariances, where phenotypic variance was estimated as $\hat{\sigma}_a^2 + \hat{\sigma}_e^2$, where $\hat{\sigma}_a^2$ and $\hat{\sigma}_e^2$ are variance estimates for additive genetic effects and independent residual term, respectively.

$$\hat{r}_g = \frac{\hat{\sigma}_{p_{t1,t2}}}{\sqrt{\hat{\sigma}_{p_{t1}}^2 . \hat{\sigma}_{p_{t2}}^2}} \quad [6]$$

The error in vector **e** was partitioned into spatially correlated (**ξ**) and uncorrelated (**η**) residuals. We have modelled **ξ** by using a first-order separable autoregressive process in the row and column directions, as suggested by (Gilmour et al., 1997) for agricultural trials, as well as by Costa e Silva et al. (2001) and (Dutkowski et al., 2002) for forest genetic trials. In this sense, the variance-covariance matrix defined for **ξ** in **R** included two autocorrelation parameters (i.e. for the row and column directions) and one variance parameter (i.e. the variance of the trend process).

Narrow-sense heritabilities after adjustment for spatial variability were estimated for each trait using $\hat{\sigma}_a^2/(\hat{\sigma}_a^2 + \hat{\sigma}_e^2)$. Estimation of the standard errors for the heritability and additive genetic correlation estimates were based on approximations using Taylor series expansion (Lynch, 1998) in ASReml.

Estimation of genetic gains

A number of selection scenarios were explored in order to estimate the genetic gain.

First, a backwards selection scenario was simulated in which parents were sorted from highest to lowest based on the breeding values (BVs) for each trait. The genetic gain (%) compared with the population mean was estimated from direct selection of each trait. Statistics obtained were: average of 10 top parents, average of 20 top parents, average of 30 top parents etc up to the average of 100 top parents.

Second, we looked at a combination of selection on merit, basically a forward selection scheme – i.e. the best 100 individual, the best 40 individuals, the best 20 individuals. We combined this with an option to maintain diversity – i.e. selecting the best individual in each family.

In all cases genetic gain was predicted as,

_____

where is the mean predicted breeding value of selected population and

is the predicted mean site mean (fixed effect) from the model.

Results

Basic statistics

The means, ranges and coefficients of variation (CV) for tree height at age 1 year, tree DBH, height, needle $\delta^{13}C$, stem straightness and malformation at age 7.5 years are shown in Table 2. It was found that coefficients of variation were larger for stem quality traits (straightness and malformation) than growth traits (height and DBH) and needle $\delta^{13}C$ (Table 2). The overall mean for the needle $\delta^{13}C$ at this dry site was -25.89‰, with a coefficient of variation of 2.6%. The range between minimum $\delta^{13}C$ (-28.34‰) and maximum $\delta^{13}C$ (-23.25‰) found among the individual trees in this breeding trial was large, which was more than 5‰.

Table 2: Means, standard errors (SE), coefficients of variation (CV), minimum (Min) and maximum (Max) for growth, needle $\delta^{13}C$ and stem quality traits measured from radiata pine trees grown at a dry site near Christchurch.

Traits	Age	Mean	SE	CV (%)	Min	Max	N
Height (mm)	1	746.28	1.96	16.80	130.00	1150.00	4100
DBH (mm)	7.5	183.95	0.41	11.63	93.00	260.00	2698
Height (m)	7.5	9.14	0.02	8.77	4.10	11.60	2675
$\delta^{13}C$ (‰)	7.5	-25.89	0.01	2.58	-28.34	-23.25	2265
Straightness	7.5	5.99	0.04	30.86	1.00	9.00	2669
Malformation	7.5	5.57	0.05	45.38	1.00	9.00	2670

Heat maps (Appendix 2-6) give graphical representations of the data at the site, for tree DBH, height, needle $\delta^{13}C$, stem straightness and malformation assessed at age

7.5 years respectively. The maps indicate that there was no particular area in the trial where trees were taller, fatter, or more malformed, for example, giving us confidence in the uniformity of the site.

Genetic parameters

Variance components and individual narrow-sense heritability estimates for the six traits are presented in Table 3. The traits we measured showed variable levels of genetic control, with heritability estimates ranging from 0.09 to 0.40 at this dry site (Table 3). The narrow-sense heritability estimates were very low for stem malformation (0.09), low for stem straightness (0.16), tree height (0.12-0.16) and DBH (0.19-0.20), but moderate for needle $\delta^{13}C$ (0.37-0.40) (Table 3). Fitting an additional spatial component to the residuals (i.e. spatial analysis) didn't improve the estimation of additive genetic variance and heritability estimates for all traits (Table 3). This indicated that the site was relatively homogeneous and the proportion of the residual variance that was spatially correlated was low.

Table 3. Estimates of variance components (VC), standard errors (SE) and narrow-sense heritability (h^2) for the traits of tree growth, stem quality and needle $\delta^{13}C$ measured from radiata pine trees grown at a dry site near Christchurch.

		Non-spatial analysis [a]			**Spatial analysis** [b]		
Trait	**Effect**	**VC**	**SE**	**h^2**	**VC**	**SE**	**h^2**
Height (mm)	Tree	2251.5	422.8		2284.2	415.4	
(Year 1)	Rep [a]	640.3	231.5		-147.7	45.2	
	Rep*Set	518.1	139.7		409.4	1302	
	Residuals	12518.0	413.5		11912.0	407.8	
	AR residual				23188.0	12157.0	
	AR1 (rows) [b]				0.99		
	AR1 (columns)			0.15±0.03	0.99		0.16±0.03
DBH (mm)	Tree	86.4	18.2		87.9	18.3	
(Year 7.5)	Rep	12.8	5.8		2.1	2.8	
	Rep*Set	6.7	3.6		4.7	3.3	
	Residuals	362.9	16.6		361.6	16.6	
	AR residual				292.6	176.4	
	AR1 (rows)				1.0		
	AR1 (columns)			0.19±0.04	1.0		0.20±0.04
Height (m)	Tree	0.06	0.02		0.06	0.02	
(Year 7.5)	Rep	0.05	0.02		-0.01	0.00	
	Rep*Set	0.06	0.01		0.03	0.01	
	Residuals	0.48	0.02		0.46	0.02	
	AR residual				3.75	1.10	
	AR1 (rows)				1.00		
	AR1 (columns)			0.12±0.03	1.00		0.12±0.03
Mal [c]	Tree	0.54	0.22		0.54	0.21	
(Year 7.5)	Rep	0.12	0.06		0.04	0.04	
	Rep*Set	0.05	0.04		0.02	0.04	
	Residuals	5.74	0.23		5.73	0.23	

	AR residual				2.99	2.14	
	AR1 (rows)				1.00		
	AR1 (columns)			0.09±0.03	1.00		0.09±0.03
Stra [d]	Tree	0.53	0.13		0.45	0.17	
(Year 7.5)	Rep	0.05	0.02		0.04	0.03	
	Rep*Set	0.01	0.02		0.00	0.03	
	Residuals	2.90	0.13		2.35	0.73	
	AR residual				0.14	0.18	
	AR1 (rows)				0.46		
	AR1 (columns)			0.16±0.04	0.17		0.16±0.05
$\delta^{13}C$	Tree	0.15	0.03		0.16	0.03	
(Year 7.5)	Rep	0.01	0.01		0.01	0.01	
	Rep*Set	0.01	0.00		0.01	0.00	
	Residuals	0.27	0.03		0.24	0.04	
	AR residual				0.04	0.03	
	AR1 (rows)				0.50		
	AR1 (columns)			0.37±0.07	0.33		0.40±0.08

[a] Rep – replication; [b] AR1 (rows), AR1 (columns) - two autocorrelation parameters as a row/column measure of spatial autocorrelation in spatial analysis; [c] Mal – stem malformation. [d] Sta – stem straightness.

Trait-trait correlations

At age 7.5 years, the phenotypic correlation between tree height and DBH was strong, while the correlation between tree height and stem malformation or straightness was moderate (Table 4, Figure 3). The phenotypic correlation between tree DBH and stem malformation or straightness was low (Table 4). However, there were only low phenotypic correlations between needle $\delta^{13}C$ and tree growth (height and DBH) or stem quality (malformation and straightness) traits (Table 4). The height-height phenotypic correlation between year 1 and year 7.5 was low, while the phenotypic correlation between tree height at year 1 and DBH at year 7.5 was moderate (Table 4).

Table 4. Genetic (below diagonal) and phenotypic (above diagonal) correlations among the traits measured from radiata pine trees grown at a dry site near Christchurch.

Trait	**Height** (Yr 1)	**DBH** (Yr 7.5)	**Height** (Yr 7.5)	**Mal** (Yr 7.5)	**Stra** (Yr 7.5)	**$\delta^{13}C$** (Yr 7.5)
Height (Yr 1)	**1**	0.31 ± 0.02	0.19 ± 0.02	-0.07 ± 0.02	-0.09 ± 0.02	0.04 ± 0.02
DBH (Yr 7.5)	0.22 ± 0.13	**1**	0.50 ± 0.02	0.08 ± 0.02	0.11 ± 0.02	0.18 ± 0.02
Height (Yr 7.5)	0.15 ± 0.16	0.39 ± 0.16	**1**	0.30 ± 0.02	0.31 ± 0.02	0.09 ± 0.02
Mal (Yr 7.5)	-0.34 ± 0.17	-0.19 ± 0.19	0.20 ± 0.21	**1**	0.42 ± 0.02	0.02 ± 0.02
Stra (Yr 7.5)	-0.17 ± 0.14	0.16 ± 0.16	0.17 ± 0.18	0.71 ± 0.13	**1**	0.01 ± 0.02
$\delta^{13}C$ (Yr 7.5)	0.08 ± 0.14	0.43 ± 0.13	0.22 ± 0.17	-0.15 ± 0.19	-0.09 ± 0.16	**1**

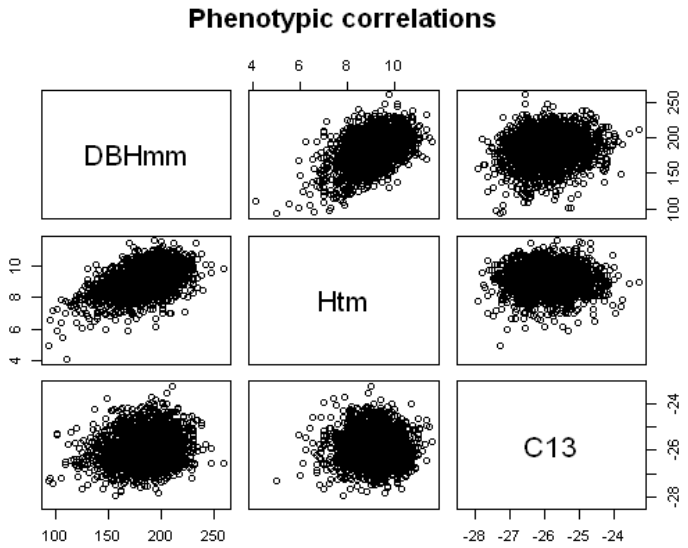


Figure 3. Phenotypic distributions among the traits of DBH (DBHmm), height (Htm) and needle $\delta^{13}C$ (C13) measured at age 7.5 years from radiata pine trees grown at a dry site near Christchurch.

At age 7.5 years, the genetic correlation was strong between stem malformation and straightness (Table 4), and moderate between tree height and DBH (Table 4, Figure 4). The genetic correlation was negative (-0.19) between tree DBH and stem malformation, but positive (0.16) between tree DBH and stem straightness. The genetic correlation between tree height and stem malformation or straightness was low but positive (Table 4). A moderate positive genetic correlation was observed between needle $\delta^{13}C$ and tree DBH. However, only a low positive genetic correlation was observed between needle $\delta^{13}C$ and tree height (Table 4, Figure 4). The positive correlations between the traits indicate that faster growth was genetically associated with higher needle $\delta^{13}C$ values.

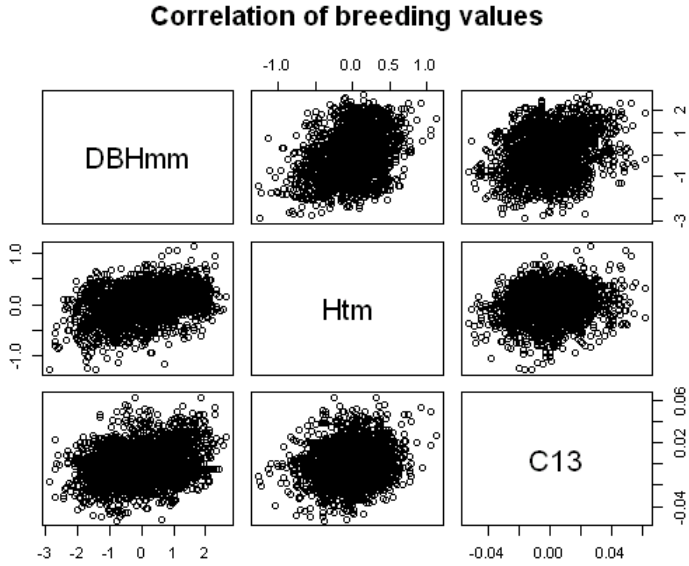


Figure 4. Correlations among the estimated individual-tree breeding values for DBH (DBHmm), height (Htm) and needle $\delta^{13}C$ (C13) measured at age 7.5 years from radiata pine trees grown at a dry site near Christchurch.

Genetic gains

Genetic gain estimated using backward selection (Figure 5) indicated that the best genetic gain at this site would be obtained from intensive selection for height (Htm). Gain estimates for backward selection for both DBH and $\delta^{13}C$ were small.

Gain estimates using several selection scenarios were also highest for height (Figure 6). Gain estimated for $\delta^{13}C$ and DBH were very small at this site.

The relatively small gain estimates is an indication that the selection intensity is reasonably low, i.e. that 120 families at one site is not a normal selection scenario, and many more site assessments are usually involved. The small gain estimates here are therefore not necessarily an indication that selection within the RPBC breeding population would not provide adequate gains.

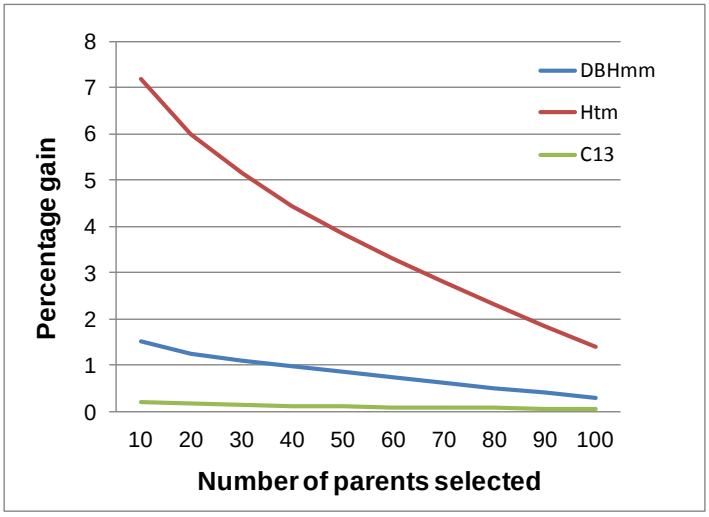


Figure 5. Predicted genetic gains from backward selection of the best parents when compared with the site mean for the key traits of DBH (DBHmm), height (Htm) and needle $\delta^{13}C$ (C13) measured at age 7.5 years from radiata pine trees grown at a dry site near Christchurch.

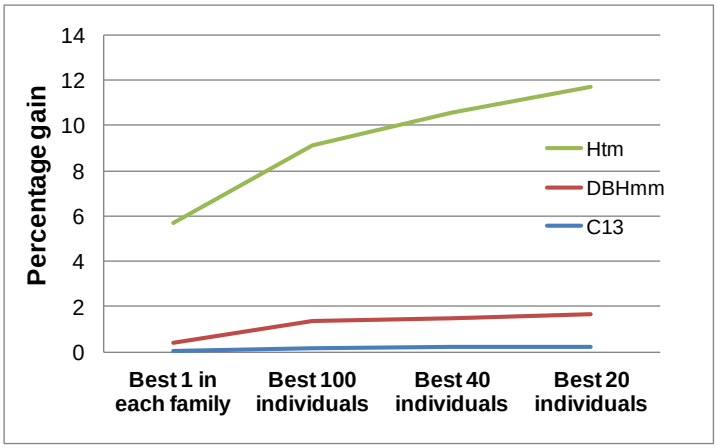


Figure 6. Predicted genetic gain, expressed as the percentage from the trial mean for different scenarios for the key traits of DBH (DBHmm), height (Htm) and needle $\delta^{13}C$ (C13) measured at age 7.5 years from radiata pine trees grown at a dry site near Christchurch

Discussion

Genetic variation and correlation of tree growth and stem quality

In New Zealand, a number of traits are routinely assessed in radiata pine progeny trials and clonal trials. They are DBH, straightness, branch cluster frequency, malformation, acceptability and wood density (Jayawickrama, 2001; Burdon et al., 2008).

The analysis indicates that the radiata pine breeding trial tested in this study had a significant amount of additive genetic variation, resulting in moderate to low narrow-sense heritabilities for growth and form traits measured. The results demonstrated useable genetic variation in tree growth and stem quality traits, as well as a clear difference in height, DBH, straightness and malformation of individual genotypes tested in this study. Genetic trials of radiata pine in New Zealand and New South Wales have shown similar heritabilities for growth and form as observed in this study (Jayawickrama, 2001). The within-site heritabilities have been reported from 0.13 to 0.27 (overall 0.19) for DBH, 0.22 to 0.27 (overall 0.27) for height, 0.20 to 0.27 (overall 0.22) for straightness, 0.05 to 0.09 (overall 0.10) for malformation (Jayawickrama, 2001). Higher heritabilities (0.23-0.55) for stem straightness have been observed for radiata pine grown a different sites across Australia (Gapare et al., 2012). Similar to previous studies, the heritability estimates were lower for stem malformation than for other traits in this study, indicating a higher environmental influence for this trait.

Genetic correlations of both straightness and malformation with DBH were favourable, which means that gains in each could be achieved through correlated response to selection on diameter alone. The practical implication of this is that modest correlated response of straightness and malformation can probably be anticipated with further selection on diameter growth.

Phenotypic and genetic variation of needle $\delta^{13}C$

We have demonstrated for the first time in a radiata pine breeding trial in New Zealand significant phenotypic and genotypic variations in needle $\delta^{13}C$. The needle $\delta^{13}C$ values varied over a range (maximum – minimum) of -5.09‰ (equivalent to 5.32‰ of $\Delta^{13}C$) among individual trees, indicating a large phenotypic variation. The range found in this study was larger than, and comparable with those reported previously for other coniferous species (Johnsen et al., 1999; Xu et al 2000, 2003; Brendel et al., 2002; Prasolova et al., 2001, 2003). Using Equation 2 and 3, the measured $\delta^{13}C$ values could be transformed into a range of WUE_i of 60-113 μmol CO_2 mol^{-1} H_2O, indicating up to 88% variation of WUE_i among the individual trees in this study. These values are generally in agreement with the values (67-100 CO_2 mol^{-1} H_2O) reported previously for maritime pine (Brendel et al., 2002), but larger than those for the clones of pedunculate oak (Brendel et al., 2008).

Our results show significant genetic variation in needle $\delta^{13}C$, with a moderate narrow-sense heritability, which was higher than those for tree height and DBH (Table 3). Similarly, narrow-sense heritability estimates by Johnsen et al. (1999) for *P. mariana* are also lower for diameter growth (0·14) than for $\delta^{13}C$ (0·54). Heritability represents the proportion of the genetic variance relative to the phenotypic variance of a trait surveyed over a large number of individuals. A high heritability not only indicates a

greater potential for a trait to respond to natural selection (Arntz and Delph; 2001), but it also means that the trait is less affected by environmental sources of variation and thus facilitates the genetic dissection of the trait (Brendel et al., 2008). Genetic variation and narrow sense heritabilities for $\delta^{13}C$ or Δ have been estimated for a number of tree species. Low heritability values of 0.17 for *P. pinaster* (Brendel et al. 2002) and of 0.20 for a dry and 0.33 for a wet environment for *A. cunninghamii* (Xu et al. 2003) have been reported. Medium heritabilities ranging from 0.15 to 0.52 have been estimated for different populations of *C. sativa* (Lauteri et al. 2004) and 0.54 for *P. mariana* (Johnsen et al., 1999), whereas high heritabilities of 0.72 were found for *A. cunninghamii* (Prasolova et al. 2001). Compared with the values reported in literature, the heritability estimated for needle $\delta^{13}C$ in *P. radiata* in this study was medium. An explanation for the moderate heritability of $\delta^{13}C$ found in this study could be related to the dry condition. The present study was located in the east Canterbury, New Zealand, where summer drought is common (Mullan et al., 2005). It has been reported that water stress could reduce the heritability of $\delta^{13}C$ (Johnson et al., 1990; Ehdaie and Waines, 1994).

Genetic correlations between needle $\delta^{13}C$ and tree growth

This study revealed that there existed low phenotypic, but moderate and positive genetic correlations between the needle $\delta^{13}C$ and growth traits (Table 4). Faster growing radiata pine trees (i.e. genotypes) at this dry site had higher WUE_i (assessed by $\delta^{13}C$) in this study, as reported previously for other tree species (Johnsen et al., 1999; Prasolova et al., 2001, 2003; Xu et al., 2003). This could be related to a higher photosynthetic capacity in needles of fast-growing trees when compared with slow-growing trees. The moderate and positive genetic correlation indicates that photosynthesis could be a main physiological component of growth performance for the individual trees (i.e. genotypes) in this breeding population. Assuming a positive correlation between photosynthetic capacity and growth, a positive correlation between $\delta^{13}C$ and growth suggests a predominantly assimilation rate-based control of $\delta^{13}C$ (Farquhar et al., 1989a). If $\delta^{13}C$ were to be controlled by stomatal conductance, the Farquhar model predicts a negative correlation between $\delta^{13}C$ and growth. Therefore, the positive correlation between $\delta^{13}C$ and growth suggests that the variation of WUE_i (assessed by $\delta^{13}C$) among the measured trees was rather controlled by assimilation (i.e. photosynthetic capacity) than by stomatal conductance. The differences of $\delta^{13}C$ among families within provenances of *P. mariana* (Mill.) Britton have been found to be mainly determined by differences in photosynthetic capacity (Johnsen and Major, 1995; Major and Johnsen, 1996; Johnsen et al., 1999). Similar results have been reported for families of *P. pinaster* (Ait.) (Guehl et al., 1995), families of *A. cunninghamii* Ait. ex D. Don (Prasolova et al., 2001; Xu et al., 2003), and the clones of the F1 hybrid between *P. elliottii* Engelm x *P. caribaea* Morelet growing in a drought-prone environment (Xu et al., 2000; Prasolova et al., 2003).

Any potential for a change in tree growth in response to selection based on $\delta^{13}C$ is dependent on the strength and direction of any genetic correlation between $\delta^{13}C$ and tree growth. In this study, moderate and positive genetic correlations were observed between needle $\delta^{13}C$ and tree growth traits. In addition, there were moderate heritability estimates (0.37-0.40) for needle $\delta^{13}C$ (Tables 3). These suggest the possibility of selecting for high water use efficiency (WUE) with the potential for simultaneous gains in height and diameter growth. This study highlights the potential of using needle $\delta^{13}C$ as a useful trait for indirectly selecting radiata pine genotypes with improved WUE as reflected in needle $\delta^{13}C$ and better tree growth under dry conditions.

Genetic gains

Genetic gains for growth traits and $\delta^{13}C$ were found to be relatively small. We believe this is due to the small selection intensity (i.e. assessment at one site) and the fact that the weather in Rolleston over the previous summer had been unseasonably wet. Gains in $\delta^{13}C$ that are relatively modest will translate to greater gains in WUE, as described above. We have faith in the fact that the heritability for $\delta^{13}C$ was moderate, and we believe selection for this trait will deliver more drought-resistant genotypes.

Implication of using foliar $\delta^{13}C$ to screen drought tolerant radiata pine genotypes in future breeding programs

The effectiveness of selection programs for drought tolerance based on tree growth alone has been relatively low because of the large number of genetic and environmental factors involved in the regulation of tree growth (Acevedo, 1993; Xu et al., 2000). In New Zealand, a large proportion of the plantation estate is located in environments where water limits tree growth and more droughts are expected with climate change (Mullan et al., 2005). Thus, there is a need to consider physiological traits such as WUE ($\delta^{13}C$ as surrogate index) in the selection of radiata pine families or genotypes with improved growth and adaptation to drought-prone environment. Physiological traits, such as $\delta^{13}C$, can be used as indirect selection criteria for tree growth in drylands. However, their effectiveness depends on their correlations with tree growth under drought and the degree to which each trait is genetically controlled. It is commonly recognised that if physiological traits are to be useful in breeding, they must have a greater heritability than for yield, a significant correlation with growth, a casual relation with yield, and physiological assays that are easy to use (Acevedo, 1993).

In this study, the genetic variances and heritabilities for needle $\delta^{13}C$ (surrogate index of WUE) were generally moderate and larger than those for growth traits (height and DBH). This indicates the genetic variation exists within radiata pine for this trait ($\delta^{13}C$) that can then be exploited in breeding programs. There were also moderate and positive genetic correlations between $\delta^{13}C$ and tree growth, indicating that breeding radiata pine genotypes for improved WUE would not necessarily come at the expense of productivity. These results highlight the potential use of needle $\delta^{13}C$ in indirect selection of radiata pine genotypes for better growth on water-limited sites. Although most physiological processes are difficult to measure quickly and so cannot be quantified in a timely fashion for large breeding or screening populations, $\delta^{13}C$ measurements are an exception. Although foliar samples collected for $\delta^{13}C$ analysis must be standardized by crown position and age class, sample collection and preparation is relatively simple and rapid, enabling the analysis of many individuals over a short period of time, making this a potentially powerful approach for understanding genetic variation in WUE and underlying physiological mechanisms in large populations.

In this study, the genetic correlations between needle $\delta^{13}C$ and tree growth were favourable, which means that gains for growth could be achieved through correlated response to selection on needle $\delta^{13}C$ alone. The practical implication of this is that modest correlated response of DBH and height can probably be anticipated with further selection on high needle $\delta^{13}C$. The results found in this study demonstrated moderate possibilities for improvement of radiata pine WUE (and growth). Such

information would be very useful for selecting drought tolerant germplasm for drought-prone areas.

Despite the potential limitations of the $\delta^{13}C$ technique under field conditions, it has been successfully applied in Australia to develop higher yielding wheat cultivars for dryland conditions. Rebetzke et al. (2002) described screening of plants in the greenhouse under well-watered conditions for high $\delta^{13}C$. Field tests of superior $\delta^{13}C$ lines from the breeding program and selection based on $\delta^{13}C$ measurements and yield resulted in the selection of a genotype that had an average yield increase of 11%. The cultivar 'Drysdale' developed in that study has been released for production in dryland areas. Previous studies have also reported on the usefulness of $\delta^{13}C$ as a trait in breeding programs in trees. For example, Zhang et al. (1996) suggested that $\delta^{13}C$ could be used as a marker for breeding programs to improve growth of *P. menziesii* and *L. occidentalis*. Similarly, Sun et al. (1996) concluded that it would be possible to use $\delta^{13}C$ as a selection trait for WUE and select *P. glauca* genotypes for high WUE without compromising yield.

Based on our results, needle $\delta^{13}C$ is heritable and should respond to selection. We also estimated gains and breeding values. However, these breeding values are the first for this trait in New Zealand, were from one site at one time. They must therefore be treated with caution, as rank changes with more information across more sites would be expected. Therefore, further testing should be warranted at more sites with contrasting soil moisture conditions to ensure no trade-off between selection of WUE and productivity for the breeding population at wet sites, which should open the prospects of selecting genotypes with high WUE without affecting overall productivity across sites with different soil moisture availability.

The RPBC has genotypes tested in arid areas in Australia that would be available for further testing of the $\delta^{13}C$ methodology. We believe that application of the $\delta^{13}C$ test in these trials would give confidence to the forest industry in selection and deployment of drought-tolerant genotypes. We will liaise with the RPBC and MPI on how this might be put in place.

Conclusions and Recommendations

Significant genetic variation and moderate heritability were detected for needle $\delta^{13}C$, indicating this trait was heritable for radiata pine in New Zealand. The moderate and positive genetic correlations between the needle $\delta^{13}C$ and growth traits suggested that the variation of $\delta^{13}C$ (a surrogate index of WUE) among individual trees may be controlled by photosynthetic capacity and photosynthesis could be a main physiological component of growth performance for this genetic material. These results highlight the potential use of needle $\delta^{13}C$ as a useful trait for indirectly selecting radiata pine genotypes with improved WUE and better growth performance under dry conditions.

We recommend, in the short term, the application of the $\delta^{13}C$ technique to select water-use efficient genotypes and throughout commercial orchards and /or the RPBC production population. This will ensure key traits important to RPBC clients are not compromised. We also recommend measurement of RPBC genotypes tested in arid areas in Australia to give confidence to the forest industry that the methodology is reliable. We will liaise with the RPBC and MPI on how this might be put in place.

In the longer term we recommend that elite germplasm and/or production population candidates are tested on drier sites to ensure future production population parents can be screened for water use efficiency and New Zealand is prepared for planting a larger number of drought-prone sites.

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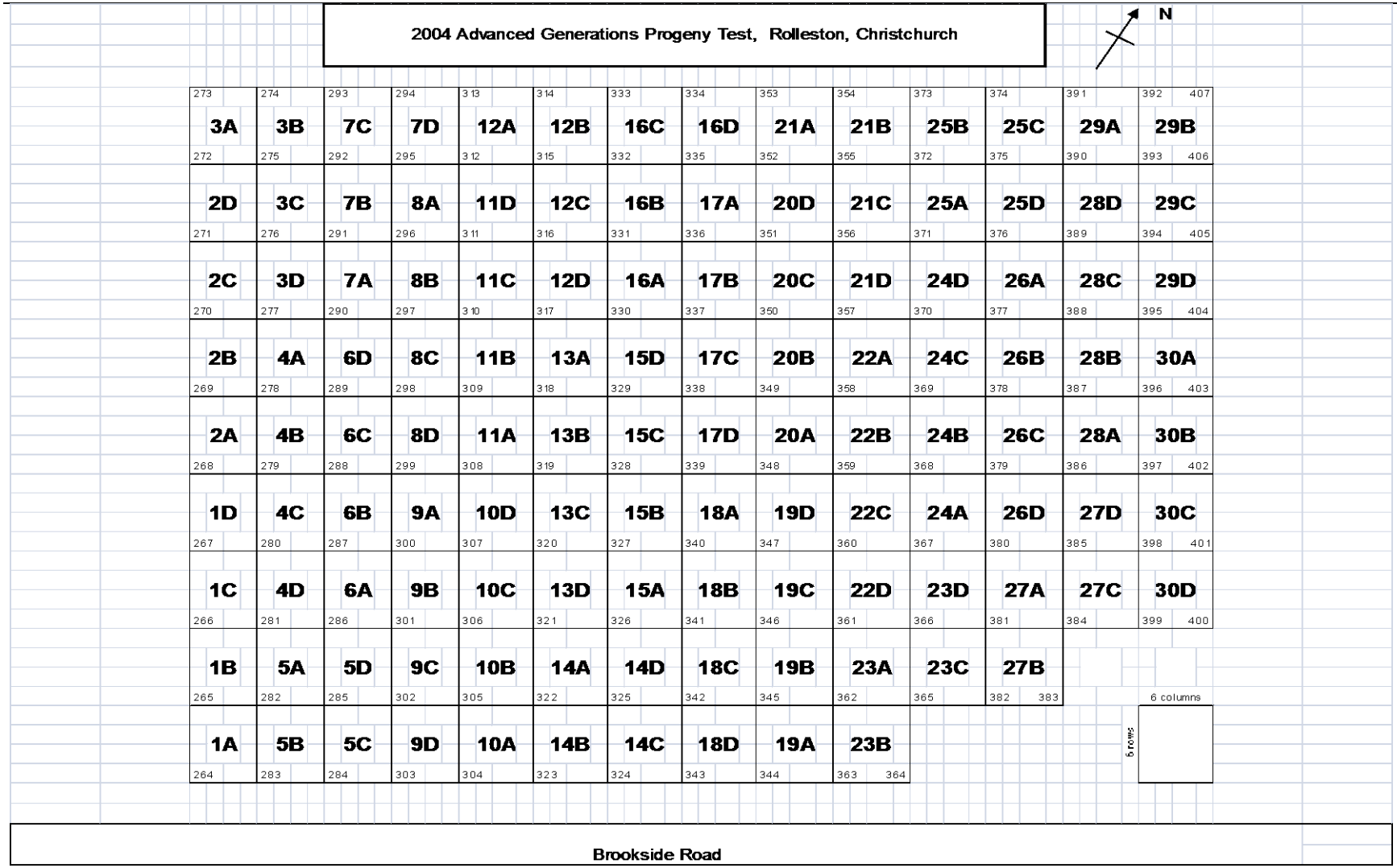
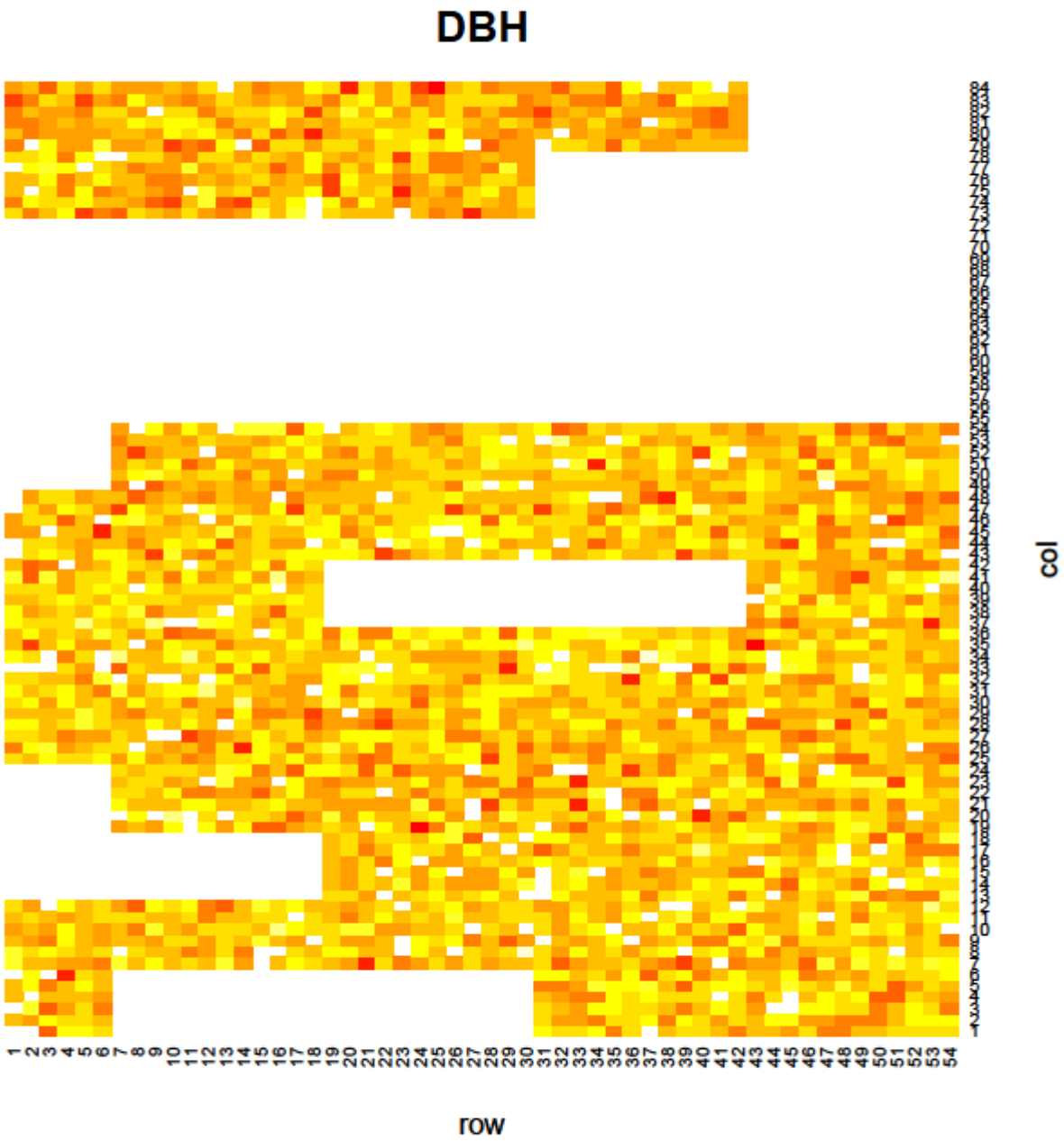
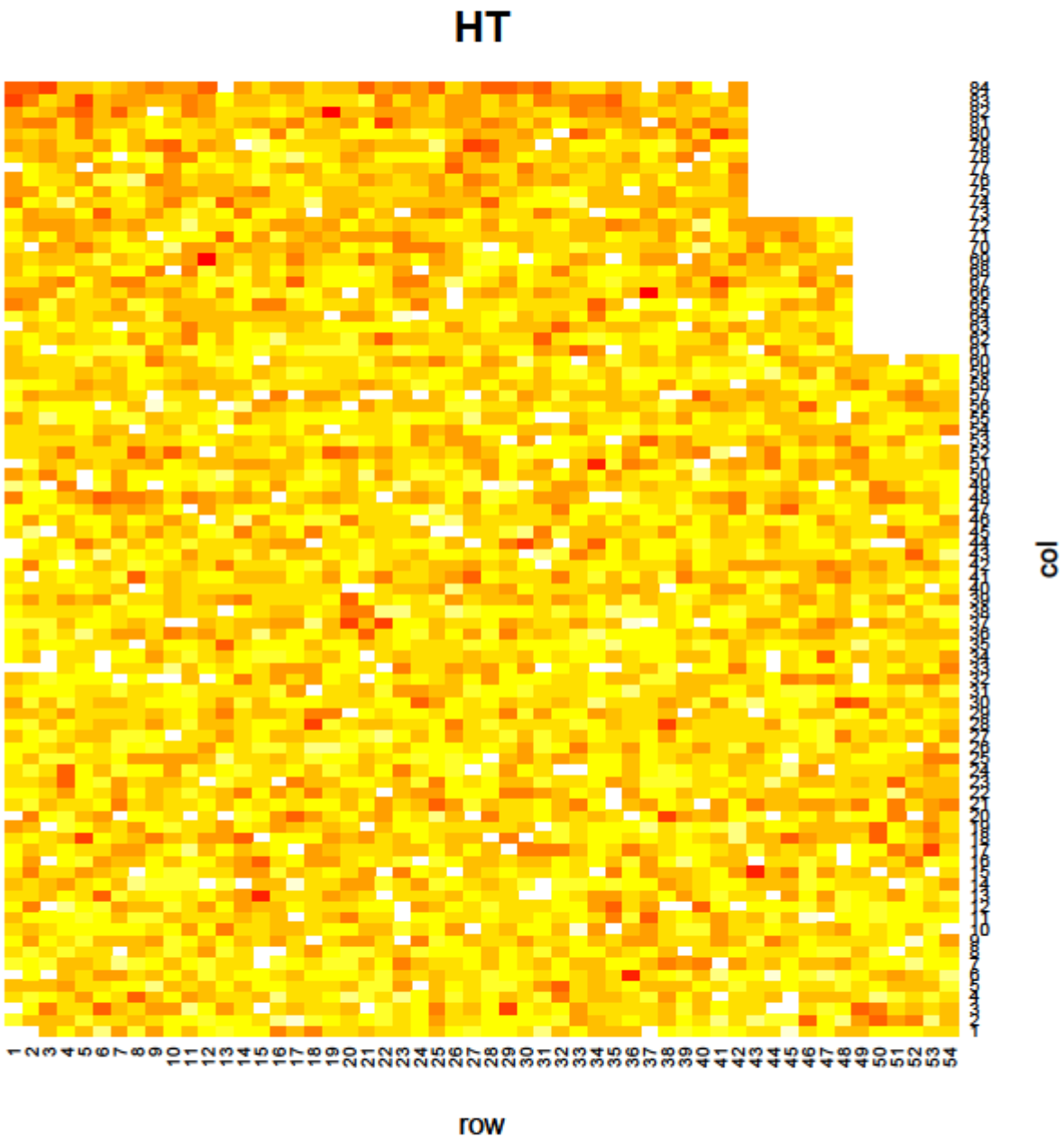
Appendix 1 2004 RPBC trial established at Rolleston, near Christchurch where measurements were undertaken for this report



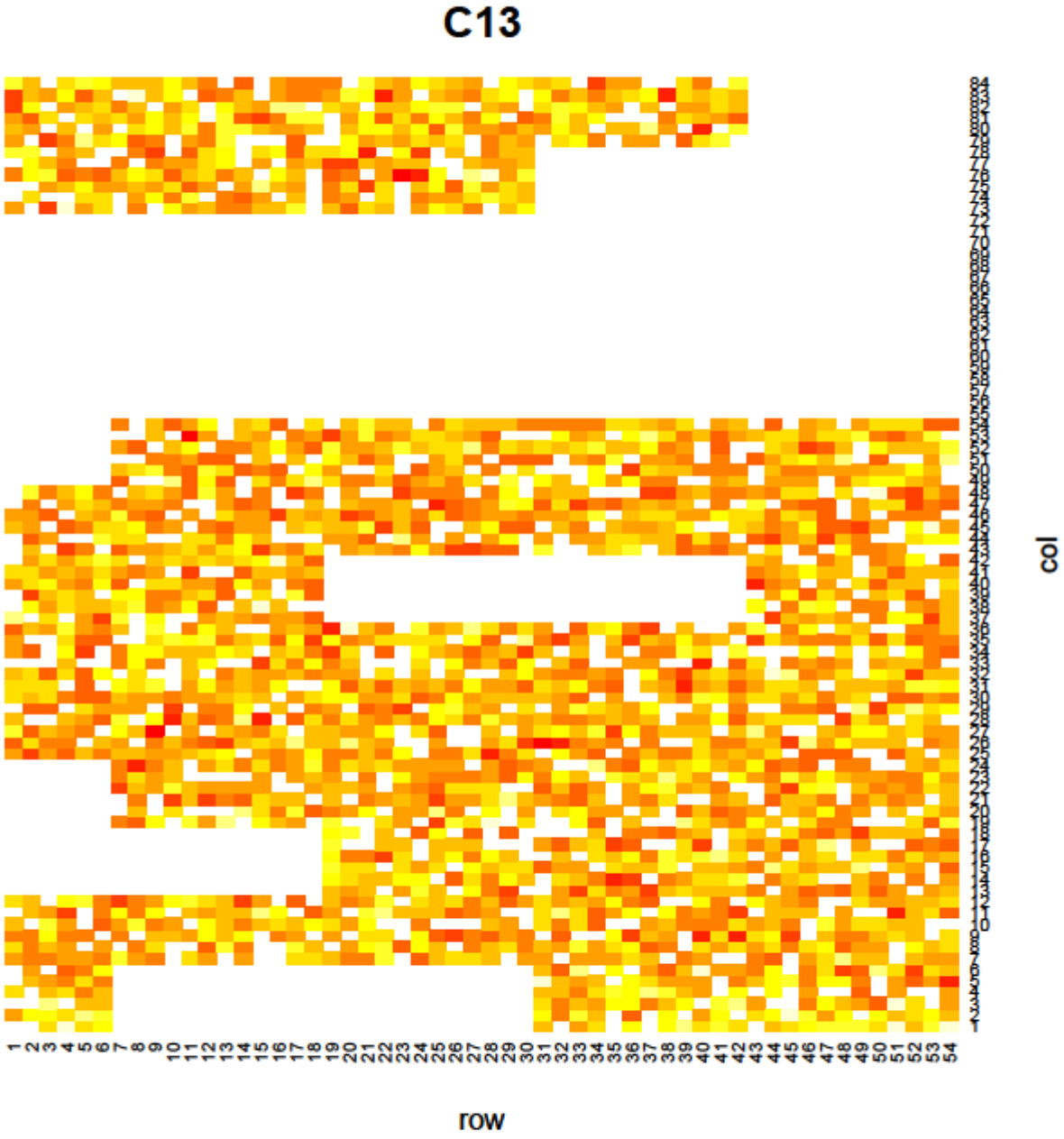
Appendix 2 Heat map for tree DBH measured at age 7.5 years



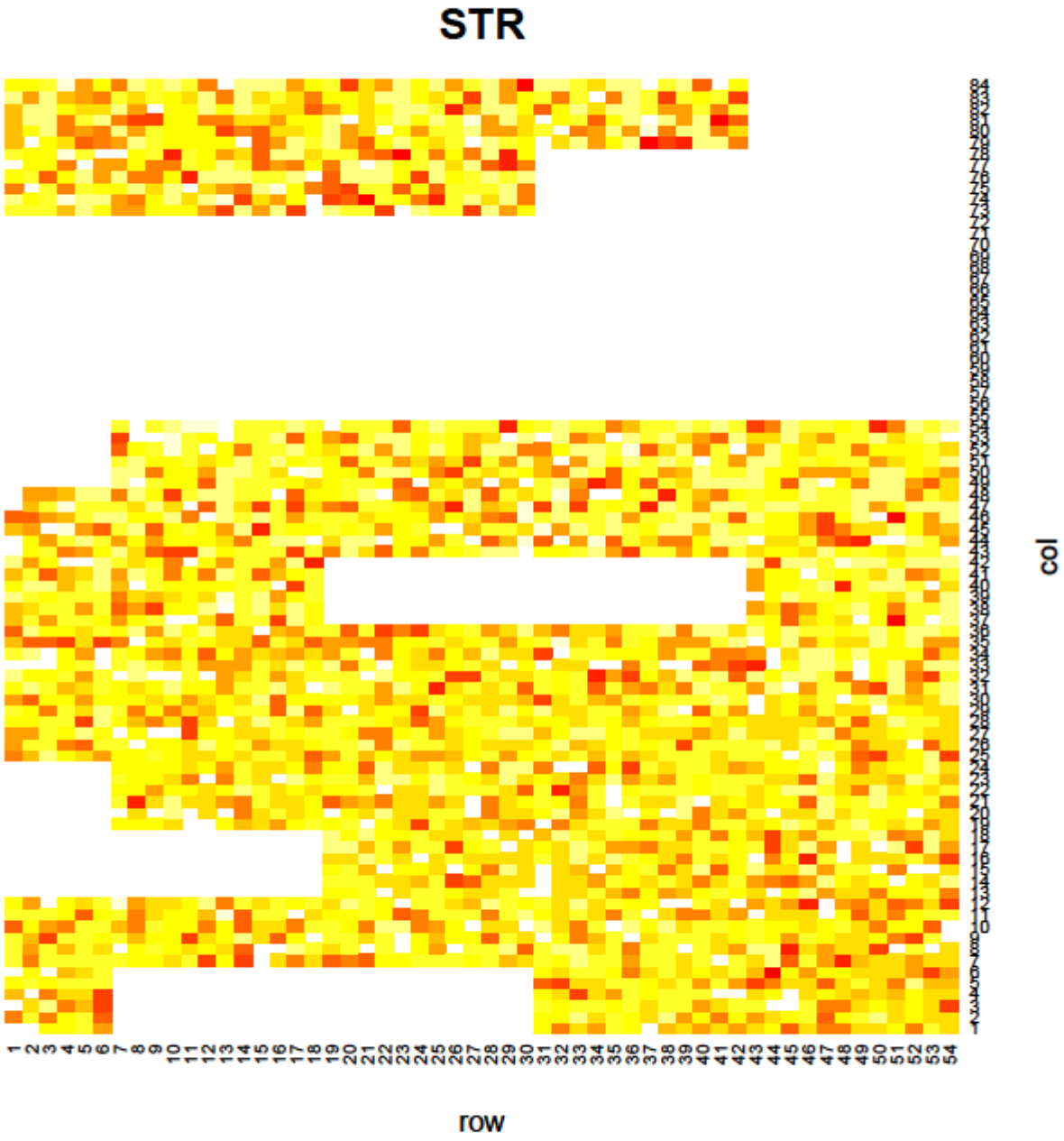
Appendix 3 Heat map for tree height measured at age 7.5 years



Appendix 4 Heat map for needle $\delta^{13}C$ measured at age 7.5 years



Appendix 5 Heat map for stem straightness measured at age 7.5 years



Appendix 6 Heat map for stem malformation measured at age 7.5 years