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Drivers of genotype by environment interaction in radiata pine as indicated by multivariate regression trees

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ABSTRACT

Productivity of forest tree plantations can be maximized by matching genetically adapted planting stock to environments where they perform best. We used multivariate regression tree (MRT) analysis with environmental predictors to quantify and characterize the nature of genotype by environment interactions (G × E) of radiata pine diameter at breast height (DBH) grown in New Zealand. The analysis was carried out for 21 provenance trials, and 48 progeny trials of second-generation selections that are widely used in plantation forestry today. To quantify the maximum variance explained by G × E, we used unconstrained clustering of genotypes based on their performance across all sites. Subsequently, the clustering was constrained by climate and soil variables, i.e. the putative causes for G × E. Unconstrained clustering explained 62% and 58% of the observed G × E variance in provenance and progeny trials, respectively. Constrained clustering explained approximately 50% and 25% of the G × E variance in provenance and progeny trials, respectively. Minimum temperature was identified as an important driver of G × E in both provenance and progeny trials. Environments can be grouped into warm humid sites, where most second-generation selected genotypes performed better, and cold sites, where specific genotypes performed best. Based on the progeny trials, only marginal (ca. 3%) gains can be made by targeted deployment to warm humid sites, but more substantial (approx. 20%) genetic gain can be made on cold sites, compared to current deployment strategies.

1. Introduction

Commercial plantations of radiata pine (*Pinus radiata* D. Don) are the basis for forest industry in New Zealand, and likewise are also important in Australia and Chile. Over the past five decades, radiata pine resources have been expanded and consolidated as a major provider of domestic and export solid-wood and pulp products. This has largely been achieved through long-term investments in tree breeding and silviculture. Significant progress has been made in understanding the genetic control of growth, form and wood quality traits of radiata pine. Based on data from genetic field trials, substantial gains of up to 32% in volume have been achieved (Mead, 2013, Table 6.3). However, an important obstacle to the realization of this genetic gain in commercial plantations lies in suboptimal matching of selected germplasm to varied environments of different regions and planting sites within regions. The New Zealand Radiata Pine Breeding Company (RPBC) programme aims to breed and provide germplasm for deployment across New Zealand, the Central and Southern Tablelands of New South Wales and Tasmania in Australia (Cullis et al., 2014). At present, RPBC produces a single set of breeding values for each trait. These breeding values are calculated including data from all available test sites, making the assumption that genotype by environment interaction (G × E) is not important.

Genotype by environment interaction (G × E) is a phenomenon in which different genotypes respond differently to variations in environment. It can consist of heterogeneous genetic variances across environments, and/or genetic correlations between expressions of a trait in different environments being low with changes of genotype rankings. The options that are available when dealing with G × E depend upon the predictability of the role of environment in generating G × E (Kang, 2002). The environments in which radiata pine grows in New Zealand have some predictable components and it is possible to exploit G × E. Johnson and Burdon, (1990), in a study of radiata pine in the New Zealand regions of Northland were able to select families for which regionalisation

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improved predicted genetic gain to 25% as compared to 22% from non-regionalisation. Carson (1991) also found an increase in expected genetic gain for diameter through regionalisation of seed orchards from 11.2% for a non-regionalised programme to 14.4% for a site-specific selection. It should be noted, however, that Johnson and Burdon's (1990) study included only four sites, whereas Carson (1991) reported findings from 11 sites with progeny derived from a set of just 25 parents. As a result, the conclusions drawn from these studies are probably insufficient to discount the need for regionalisation. Burdon et al. (1997) investigated the relative performance of three provenances from California and three land races across 21 sites throughout New Zealand. Strong differences among sources in their relative performance on different site categories were reported. A recent large G × E study by McDonald (2009) reported that genetic correlations for diameter at breast height growth between sites averaged 0.50. The study also reported evidence for heterogeneous genetic variances (i.e. level of expression) for diameter growth across different regions in New Zealand.

For multi-site progeny trials in forestry, site–site genetic correlations (also called type-B genetic correlations) were usually estimated using linear mixed models (Burdon, 1977). Since 1980s, Singular Value Decomposition (SVD) was employed to describe G × E patterns (Gauch, 1992), initially applied for agronomic crops using Additive Main effects and Multiplicative Interaction model (AMMI), and later on in forestry trials (Wu and Ying, 2004). More recently, factorial regression using a mixed model approach (Factor Analytic method) was introduced to explore G × E patterns for multi-environment trials (Smith et al., 2001; Costa e Silva et al., 2006; Beeck et al., 2010; Cullis et al., 2010) and to relate underlying factors to the causes of G × E interactions (Costa e Silva et al., 2006; Cullis et al., 2010; Hardner et al., 2011; Cullis et al., 2014). Beside linear and non-linear fixed and mixed models using parametric approaches to decompose the G × E interactions, Multivariate Regression Trees (MRT) are a method to analyse G × E that can also handle categoric as well as ordinal environmental variables (Sheaves et al., 2007; Chen et al., 2010; Hamann et al., 2011). The method has been originally developed for ecological research, to analyze interactions between environmental variables and species abundance in ecological communities (De'ath, 2002; Larsen and Speckman, 2004). The method is a recursive binary partitioning algorithm that assigns objects of the response matrix (species in inventory plots, or genotypes in genetic test plantations) to homogenous groups, with partition criteria being sourced from a separate data matrix (environmental variables for each site or plot).

In the present study, we explore whether multivariate regression tree analysis can be applied to identify and quantify environmental drivers of G × E in radiata pine grown in New Zealand. We first re-analyze archival data from previously published work (Burdon et al., 1997) which found strong G × E across a wide range of test environments, to investigate if the technique can reliably replicate these results using a recursive algorithm to identify the primary environmental drivers of G × E. Second, we applied the MRT analysis to a large data set from 48 second- and third-generation radiata pine progeny trials established by the RPBC, with some of the genotypes in these trials widely deployed in New Zealand for commercial plantation forestry. The main objective of this paper is to investigate if we can identify environmental drivers of G × E that could be translated into straightforward guidelines to plant particular sets of genotypes under different planting-site environments. Lastly, this study contributes a broad comparison of how unimproved provenances or land races (Burdon et al., 1997) compare to genetically improved second- and third-generation selections (RPBC material) in their response to different environments.

2. Materials and methods

2.1. Radiata pine provenance trial data

Twenty-one provenance trials planted across New Zealand provide the experimental basis for the first part of this study, site information being provided in Table 1. Seventeen trials represented provenances as 6-tree row plots with 12 replicates in randomized complete blocks. At the sites Pouto, Riverhead, Rotoehu, and Kaingaroa, 6 × 6-tree plots with 10 replicates were used, and 6 × 6-tree plots with five replicates were used at the sites Berwick and Longwood. Tree spacing varied among trials with 16 trials with a typical spacing of 4 × 3 m (see Table 1 for details).

Three seed origins from California, as well as three land races of naturalized New Zealand sources were planted at all provenance trials. California collections of radiata pine (Eldridge, 1978) included an average of 40 seed parents from each of 13 local subpopulations but are analyzed here as three main populations Año Nuevo (four localities), Monterey (six localities) and Cambria (three localities). The breakdown into subpopulations was disregarded, as previous studies reported subpopulation differences being negligible (Burdon et al., 1992, 1997; Raymond and Henson, 2009). The three regional land-race stocks, Kaingaroa, Nelson and Southland were collected mostly from select-trees found in unimproved stands (Burdon et al., 1997). The landrace seedlots were representative of 15 stands in Kaingaroa, six in Southland, and one large commercial stand in Nelson (Burdon et al., 1997). For simplicity, we refer to all genetic entries as provenances.

Assessments were carried out when provenance plantations reached 7–10 m in height, which varied among sites and led to measurements being carried out between 5 and 15 years (Burdon et al., 1997). Diameter at breast height (DBH) was chosen as the variable to study because it has two advantages: (i) having been measured with good precision throughout, and (ii) often DBH is more sensitive to maladaptation than height growth (e.g., Rais et al., 2014). DBH data had already been subjected to spatial adjustments where possible, as in Gapare et al. (2012) for microsite differences to reduce residual error as recommended by Costa e Silva et al. (2001) and Dutkowski et al. (2002, 2006). To standardize measurements from different ages and different site types, we subtracted the mean of individual-tree DBH in cm at each test site and divided by the test site standard deviation, so that each individual-tree DBH is expressed in units of standard deviations from a site mean of zero. Best Linear Unbiased Estimates (BLUEs) were obtained for each provenance and site, treating provenances as fixed effects using the software ASReml (Gilmour et al., 2009). We note here, that standard errors were slightly larger for the land races (0.14–0.15) than for the Californian origins (0.09–0.10), for standardized BLUEs that ranged from −0.82 to +0.82. However, this should not influence the G × E analysis presented in this paper other than producing a residual error variance.

2.2. Radiata pine progeny trial data

The second dataset we used in this study contains 48 field trials of the RPBC. The trial design was in most cases, randomized complete block designs, with two trials having incomplete blocks. Most trials were planted with restricted randomization of families in disconnected sets that were randomised in main plots within replicates, and then families were randomized within sets. The numbers of plots, blocks, family-sets-in-blocks and parents varied among trials and are provided in Table 2. Thirty-three trials contained controlled-pollinated (CP) families and the remaining 15 contained open-pollinated (OP) families.

Table 1
Location, spacing, and environmental variables identified as relevant by the regression tree analysis for the radiata pine provenance trials series in New Zealand.

Trial name	Spacing (m × m)	Latitude (°S)	Longitude (°E)	Elevation (m asl)	MAP (mm)	MinTCM (°C)	Soil type	[a]Burdon et al. (1997) site categories
Aupouri	4 × 3	34°45′	173°00′	30	1207.3	8.9	Coastal sand	2
Balmoral	4 × 3	42°50′	172°45′	170	624.2	2.0	Fluvial gravel	5
Berwick	4 × 3	46°00′	170°00′	520	774.0	0	Schist loess	5
Dean	4 × 3	46°00′	167°30′	200	775.0	−0.5	Schist loess	5
Golden downs	4 × 3	41°35′	172°45′	300	1490.6	0.5	Weathered fluviogravel	4
Kaingaroa	4 × 4	38°40′	176°45′	560	1326.9	1.1	Pumice	3
Kaniere	4 × 3	42°45′	171°05′	220	2976.9	2.1	Bladed podzol	NC
Longwood	3 × 3	46°17′	167°40′	250	774.0	0.7	Schist loess	5
Mangaokewa	4 × 3	38°35′	173°20′	270	1736.5	2.7	Fine volcanic ash	3
Mohaka	4 × 3	39°17′	177°00′	420	1279.5	4.1	60 cm pumice over mudstone	4
Nemona	4.5 × 3	42°35′	171°10′	200	2829.0	2.7	Bladed podzol	NC
Ngaumu	4 × 3	41°06′	176°15′	280	1242.9	3.0	Fertile clay (mudstone)	4
Pouto	3.5 × 3.5	36°33′	174°05′	50	1138.3	6.9	Coastal sand	2
Riverhead	3.3 × 3	36°50′	174°35′	100	1418.3	6.5	Gumland clay	1
Rotoehu	4 × 4	38°00′	176°35′	150	2162.3	3.0	Pumice	3
Ruatoria	4 × 3	37°45′	178°55′	180	2015.9	5.0	Fertile loam	4
Mahana	4 × 3	41°26′	173°05′	80	1113.5	1.4	Weathered fluvioglacial gravel	1
Waimate	4 × 3	44°58′	170°55′	620	732.4	−2.9	Greywacke loess	5
Waimihia	4 × 2.4	38°50′	176°15′	760	1561.2	0.5	Pumice	3
Wairau	4 × 3	41°30′	173°30′	325	1641.3	0.3	Schist-derived loam	4
Waitangi	4 × 3	36°15′	174°00′	25	1651.3	8.0	Gumland clay	1

MAP = mean annual precipitation; MinTCM = mean daily minimum temperature coldest month.
[a] Burdon et al. site categories: 1 = Infertile clays (IC); 2 = Coastal dunes (other); 3 = Volcanic plateau (other); 4 = Central (other); 5 = Southern S.I (other); NC = no class.

A multi-environment trial analysis of the DBH measurements was conducted using a mixed model analysis with a factor analytic variance structure for the G × E effects and separate variance for the errors for each trial (e.g., Beeck et al., 2010; Cullis et al., 2010, 2014). In this analysis, the reduced animal model of Cullis et al. (2010) was extended to accommodate multi-environment trial data. The analysis was carried out by Cullis and Jefferson (2012) using ASReml-R (Butler et al., 2009). It provided best linear unbiased predictors (BLUPs) of parental additive genetic effects for each parent at each site for subsequent analysis. In the subsequent analysis we included BLUPs of 24 parents that were most widely used in commercial plantations and that were tested in all main deployment regions of New Zealand (Northland, Bay of Plenty, Central North Island, North Island East Coast, Nelson, Canterbury, and Otago-Southland).

As with most tree breeding programs, the allocation of parents to trials is incomplete, resulting in a sparse genotype by trial matrix. Of the 24 parents by 48 trial-site matrix, parents were represented on 31% of all site-parent combinations, and each parent was tested at 10 to 23 sites. The BLUPs we used in our analysis were extracted from the complete analysis by Cullis and Jefferson (2012) that used a sparse matrix. For unbalanced designs, BLUP estimates are also preferable because they converge toward the overall mean where data coverage is sparse, so that less precise estimates have less influence on G × E clustering procedures. Less precise estimates therefore drive groups to a lesser degree because they explain less variance in the cluster approach that minimizes within-group variance.

2.3. Climate and soil data

Climate data were obtained from Land and Environment New Zealand (LENZ) (http://lris.scinfo.org.nz/layers). The resolution of climate data was a 0.05° latitude/longitude grid, covering all of New Zealand. The climate data was generated with a thin-plate smoothing spline model based on latitude, longitude and elevation (Tait et al., 2006). For each provenance- or progeny-trial test site, we extracted figures for mean annual precipitation, temperature, radiation, and moisture indices as annual averages, maxima or minima where appropriate, or averages for different periods of the year, such as the warmest quarter or the wettest quarter. These variables were selected for biological relevance according to Watt et al. (2010).

Soil variables and soil classification data were also obtained from Land and Environment New Zealand (LENZ) (http://lris.scinfo.org.nz/layers) and extracted for all test sites. Soil data was provided as polygon data with a useful resolution in 1:50,000 mapping. Additional soil data, depth to slowly permeable horizon, drainage, macroporosity at depth and at surface, maximum salinity, minimum pH, phosphate retention, total carbon, potential rooting depth and topsoil gravel content were extracted from the *National Soils Database and the New Zealand Fundamental Soil Layers* (Wilde et al., 2000).

2.4. Statistical analysis

To investigate the relationship between matrices of genetic data (provenance or progeny performance at multiple sites) and environmental predictors (climate and soil variables at multiple sites), we used multivariate regression tree (MRT) analysis. MRT can be viewed as a constrained clustering, where groups with similar measurement are determined in one dataset, but the group partitioning criteria are based on a second dataset. Observations in the first dataset (genetic data) are grouped with an empirical algorithm that tests various predictor variables and cut-off values in the second dataset (environmental variables), aiming to minimize the within-group multivariate variance (De'Ath, 2002). Here, we group planting sites based on their similarity in performance of genotypes. MRT analysis was implemented with the *MVpart* package v1.2–6 for the R programming environment (R Development Core Team, 2011).

We also complemented the constrained clustering with a regular, unconstrained cluster analysis (i.e. grouping based on similarity of genotype performance only, not considering environmental variables as partitioning criteria), so that we know the maximum variance that could be explained by G × E. Hierarchical clustering was performed with *hclust* in R, using Euclidian distances and Ward's minimum variance clustering method (Ward, 1963) for

Table 2
Experimental design and environmental variables identified as relevant by the regression tree analysis for the 48 radiata pine progeny trial sites in New Zealand.

Trial ID	# Blocks	# Sets	# Plots	# Parents	Family type	Latitude (°S)	Longitude (°E)	Elevation (m asl)	MAP (mm)	MinTCM (°C)	Soil type
S1	5	9	6851	271	OP	37°06′	174°33′	62	1264	6.7	Sand
S2	10	4	3827	101	OP	36°49′	174°20′	62	1240	6.7	Sand
S3	6	5	1054	40	CP	36°49′	174°20′	62	1240	6.7	Sand
S4	10	4	4525	107	OP	37°30′	175°26′	113	1435	5.1	Sand
S5	6	6	1360	42	CP	37°30′	175°26′	113	1435	5.1	Sand
S6	35	0	1209	19	OP	38°12′	177°03′	277	1600	4.3	Gravel
S7	30	0	483	19	OP	38°28′	175°57′	147	1610	2.6	Sandy silt
S8	30	4	3075	125	CP	37°16′	174°40′	62	1289	6.7	Sand
S9	30	5	3635	157	CP	38°29′	176°16′	327	1644	2.7	Sandy silt
S10	30	5	4642	157	CP	38°33′	176°26′	521	1466	3.2	Gravel
S11	32	4	3952	128	OP	38°13′	177°02′	277	1605	4.2	Gravel
S12	25	3	1946	12	CP	38°13′	176°53′	493	1792	3.4	Gravel
S13	30	6	5176	194	CP	39°23′	177°10′	509	1710	2.1	Sandy silt
S14	30	3	1984	103	CP	38°32′	177°06′	232	1644	2.4	Loamy sand
S15	32	2	3930	34	CP	38°12′	177°10′	53	1613	4.4	Gravel
S16	30	2	900	53	CP	38°47′	176°57′	521	1498	1.9	Loamy sand
S17	30	2	1282	53	CP	38°42′	176°21′	327	1323	3.3	Sandy silt
S18	30	2	1231	49	CP	38°10′	177°25′	277	1682	3.7	Loamy sand
S19	30	0	701	47	CP	38°28′	175°13′	556	1601	3.1	Gravel
S20	30	0	823	43	CP	38°14′	176°57′	53	1575	4.1	Gravel
S21	6	10	1469	34	CP	38°17′	176°53′	493	1799	3.0	Gravel
S22	6	5	990	35	CP	38°17′	176°53′	493	1799	3.0	Gravel
S23	6	10	2008	43	CP	36°59′	174°26′	62	1212	6.9	Sand
S24	6	5	1174	35	CP	36°59′	174°26′	62	1212	6.9	Sand
S25	30	2	1437	58	CP	38°35′	176°18′	147	1411	3.4	Sandy silt
S26	5	9	2627	33	CP	38°17′	176°53′	493	1799	3.0	Gravel
S27	5	9	2035	33	CP	37°13′	174°39′	62	1286	6.7	Sand
S28	26	6	1983	112	CP	38°24′	178°26′	273	1553	3.3	Sandy loam
S29	30	6	2397	86	CP	38°23′	176°37′	556	1752	2.2	Sandy loam
S30	30	6	3321	116	CP	38°15′	175°02′	277	1574	4.2	Loamy coarse sand
S31	10	4	4116	105	OP	42°18′	173°23′	632	1660	0.2	Hill soils
S32	15	0	1327	27	CP	37°24′	176°13′	521	1405	1.7	Sand
S33	46	4	6354	105	CP	38°04′	175°13′	10	1550	4.7	Fine sandy loam
S34	45	5	6968	171	OP	38°38′	176°54′	232	2183	3.2	Gravel
S35	35	5	5754	170	OP	38°33′	176°13′	631	1528	1.5	Sand
S36	33	5	5065	169	OP	38°40′	176°02′	337	1271	3.1	Gravelly sand
S37	33	5	5043	169	OP	38°47′	176°51′	631	1541	1.6	Sand
S38	15	0	2972	27	CP	38°46′	176°50′	631	1545	1.6	Sand
S39	6	5	1452	25	CP	38°47′	176°51′	631	1541	1.6	Sand
S40	6	5	1085	40	CP	38°14′	178°26′	503	2441	3.6	Sandy loam
S41	10	4	5433	106	OP	39°02′	176°35′	631	1473	1.5	Sand
S42	50	4	8174	182	CP	39°02′	176°34′	631	1473	1.5	Sand
S43	6	6	1796	42	CP	39°02′	176°34′	631	1473	1.5	Sand
S44	5	10	8121	372	OP	38°27′	177°10′	277	1763	2.3	Gravel
S45	5	16	14,544	588	OP	39°20′	176°13′	337	1331	1.9	Sand
S46	5	10	7847	298	OP	46°09′	170°11′	119	845	0.4	Sandy loam
S47			3766	100		46°36′	170°32′	399	976	−1.4	Sandy loam
S48			1296	25		42°42′	171°44′	218	3042	2.7	Hill soils

MAP = mean annual precipitation; MinTCM = mean daily minimum temperature coldest month.

finding spherical clusters similar to MRT, implemented with the *pvclust* package for the R programming environment (R Development Core Team, 2011).

There are several methods to trim trees from constrained or unconstrained cluster analysis. The *mvpart* package provides guidance via a complexity parameter, and via variance explained by successive nodes in a screen plot, where the first nodes explain most of the variance and additional nodes have diminishing importance (similar to principal component analysis). We report variance explained by each node in constrained and unconstrained cluster analysis and subjectively trim the cluster to omit additional nodes that do not explain a substantial amount of additional variance.

3. Results

3.1. Provenance trial analysis

The first three nodes of the cluster analyses of the provenance performance across multiple sites (G × E matrix) explain 62% and 34% of the variance in the unconstrained and constrained version, respectively (Fig. 1). The bar charts at the nodes and leaves of the dendrogram represent the performance of the six provenances at a particular group of sites. For clarity, performance is expressed as deviation of each provenance from its average performance across all sites. Upward bars can therefore be interpreted as diameter at breast height (DBH) higher than average, downward bars lower than average. All variance shown in the plots therefore represents G × E. For the unconstrained clustering, the cluster analysis finds two sites, where the provenance "Cambria" significantly under-performs, explaining a third of the total variation in the G × E matrix (Fig. 1a). Notably, there is no environmental variable in our constrained cluster analysis that can separate this group of two planting sites, and this portion of the variance remains unexplained in Fig. 1b.

The second split in the unconstrained cluster analysis identifies eight sites, where Cambria shows above-average performance, explaining just under a fifth of the total variance in the G × E matrix. This is to some degree mirrored by the first split of the constrained cluster analysis that is attributed to infertile clays in the

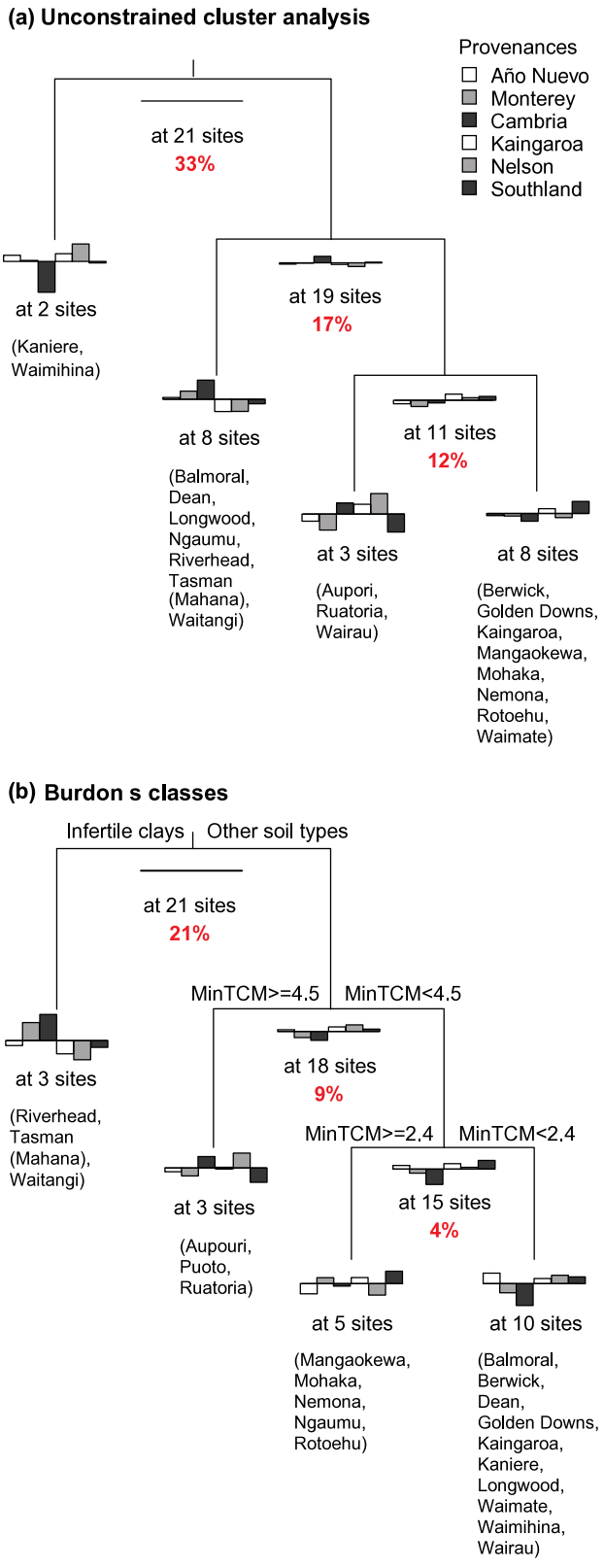

Fig. 1. Unconstrained (a) and constrained cluster (b), grouping 21 planting sites according to the performance of Año Nuevo, Monterey and Cambria provenances and three regional land-race stocks, 'Kaingaroa', 'Nelson' and 'Southland'. The same order of provenances used for legend (vertical order of listing) is also used for the histograms (horizontal order). Variances in genotypic values explained by each node of the cluster are indicated in red. In gray scale, colored bars represent group means expressed in deviation from an overall mean of zero (horizontal line). Site names are given below each group.

constrained cluster analysis: all three infertile clays sites also appear in the second cluster from the left in the unconstrained analysis. The third split of the unconstrained cluster appears near-identical to the constrained cluster, with the same three sites (Aupouri, Pouto, and Ruatoria) separated by high minimum temperature of the coldest month. An additional split in the constrained cluster analysis separates the coldest planting sites, where provenances Monterey and Cambria tend to under-perform.

In summary, we find prevalent cross-over interactions in the provenance dataset, where some provenances outperform others at one set of sites, but the reverse is true for other site types. There appears to be some correspondence between the unconstrained and constrained cluster analysis, but the unconstrained cluster analysis also revealed that important G × E exists that could not be explained by environmental data available for the test sites.

3.2. RPBC progeny trial analysis

The unconstrained clustering analysis of genotypic performance of the 24 most-tested parents at 48 RPBC progeny trials revealed less pronounced cross-over interactions (Fig. 2). Here, we provide two sets of bar charts for each group. BV refers to absolute breeding values of the RPBC breeding program, while δBV is normalized as in Fig. 1 to better visualize interactions. Parent genotypes are ordered from the highest to the lowest overall breeding value from left to right. The first split in the unconstrained analysis primarily distinguishes between high-performing parents (left two-thirds of the bar chart) that do particularly well at nine sites (Group A), whereas they somewhat under-perform in relative terms at the 39 remaining sites. Nevertheless, in terms of absolute breeding values, they still rank as top-performers at those sites. The second split is similar in principle, but Group B identifies overall high-performing parents that more severely underperform at another 16 sites.

Note that unlike in the provenance trial dataset, G × E revealed by these splits are only partially due to cross-over interactions. Also, in the progeny trial dataset, the unconstrained cluster analysis is quite closely mirrored by the constrained analysis (Fig. 3). Group A, characterized by planting sites where the best overall performers do exceptionally well in Fig. 2, overlaps with high-precipitation sites identified by the MRT analysis. Group B, the overall best-performing parents, do relatively poorly at sites with the lowest minimum temperature of the coldest month.

In summary, we find that a set of parents highlighted in gray in Figs. 2 and 3 perform disproportionately well at wet sites, and relatively poorly at cold sites. That said, the amount of variance explained by the splits in unconstrained and constrained cluster analysis differ substantially, indicating that other site factors that we did not measure have substantial contributions. The type of G × E that accounts for most of the variance does not represent cross-over interactions, where some parents outperform others at some sites with the opposite being true at other sites. Cross-over interactions do exist, however, in the second split of the constrained analysis (Fig. 3), indicating that there could be opportunity for genetic gain by deploying different parents to the coldest planting sites.

4. Discussion

4.1. Environmental variables responsible for G × E

Our analysis suggests that the likely drivers of G × E are soil factors and minimum temperature. Our first analysis with provenance

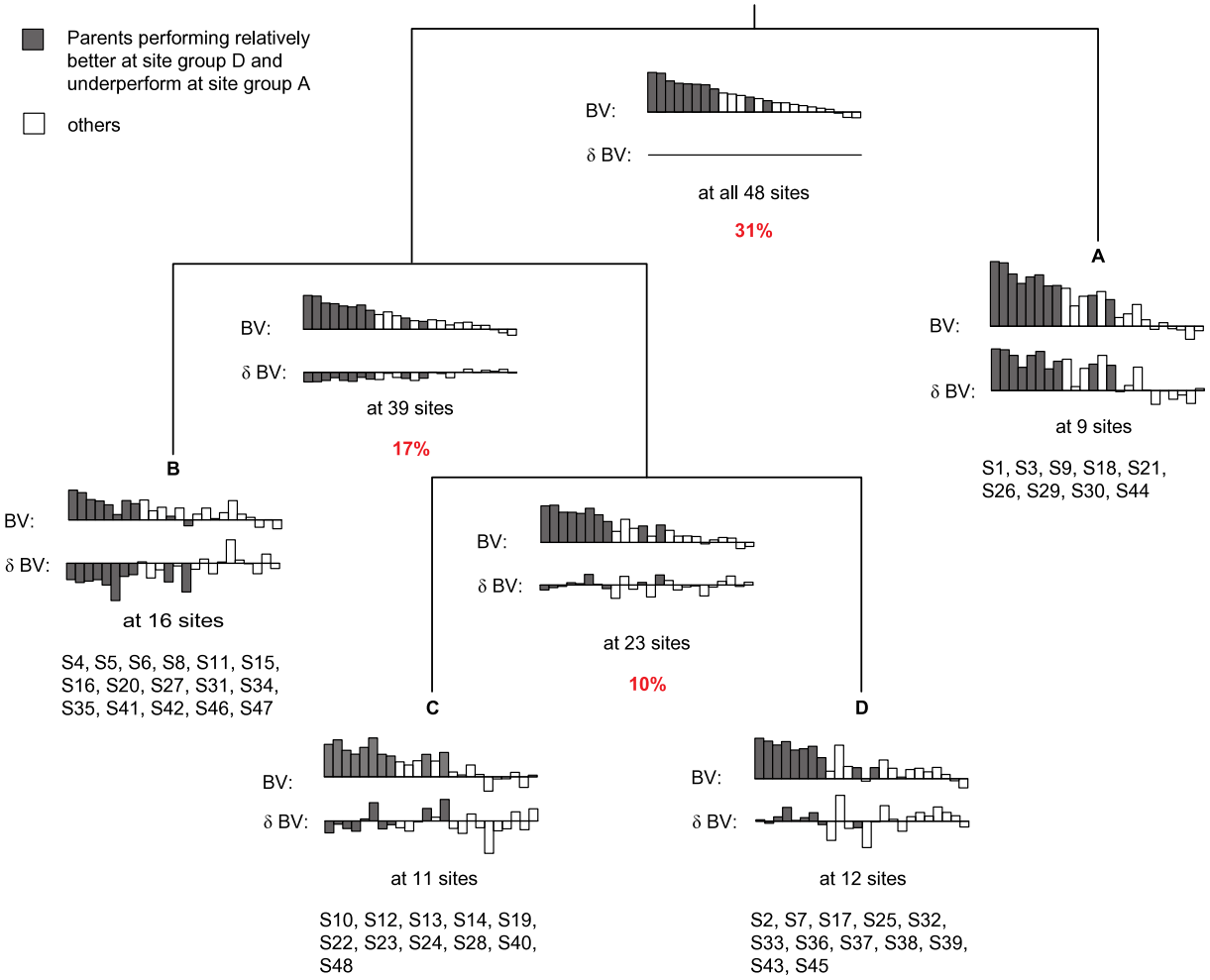

Fig. 2. Unconstrained cluster analysis, grouping 48 planting sites according to the performance of 24 parental genotypes. Genotypes are ordered from left to right by breeding value (BV) and the upper bar chart displays estimated genotypic values across a particular group of sites (BV), the lower bar chart the deviation from their average breeding values (δBV). Genotypes plotted in shading perform relatively better at site group A, but underperform at site group B. Variances in breeding values explained by each node of the cluster are indicated in red. Site codes are shown below each group.

data showed that the multivariate regression tree approach can approximately replicate the results of Burdon et al. (1997), detecting strong G × E among provenances grown on a wide range of sites across New Zealand. For example, Cambria was classified as underperforming at two sites (Kaniere and Waimihia) as was observed in Burdon et al. (1997) site classes. However, Kaniere, having a particularly high rainfall, would have been very conducive to Dothistroma needle blight, to which the Cambria provenance is especially susceptible (R.D. Burdon – personal communication). Dothistroma infection has a strong negative impact on growth. Growth losses are directly proportional to the amount of the crown that is infected. For example, Gapare et al. (2011) reported negative genetic correlations between Dothistroma defoliation and DBH growth in radiata pine grown in New Zealand. Our constrained analysis, however, could not find environmental factors that distinguished those two sites, and that could potentially account for a large portion of the variance in the unconstrained cluster analysis (Fig. 1a, left group). Our interpretation is that an environmental factor that we either did not measure, or that was not accurately enough represented in the available soil data is responsible for this interaction. The analysis also showed Cambria performing relatively better in a site cluster that includes three infertile clay sites (Mahana, Riverhead and Waitangi), also consistent with Burdon et al. (1997). In addition, Año Nuevo performed best at the coldest sites and Cambria the worst.

In the progeny-trial analysis, mean annual precipitation (MAP) and minimum temperature of the coldest month (MinTCM) were identified as the most likely drivers of G × E. Interestingly, cross-over interactions were much less prevalent in this trial series. A possible reason is that the Cambria provenance, primarily responsible for G × E in the provenance trials, was not included in the ancestry of the New Zealand breeding land races. Breeding programs regularly select for genotypes that have stable performance over a wide range of environments in addition to identifying the best performers. The second important observation from this analysis is that the type of G × E that accounts for most of the variance in the progeny trial series does not represent cross-over interactions, where some parents outperform others at some sites, while the opposite is true at other sites (Fig. 3 first split). The current practice of the RPBC to produce a single set of breeding values for each trait, making the assumption that G × E is not important, therefore appears well supported by our results for the warm humid sites.

Due to sparse nature of our data, BLUP calculations resulted in shrinkage of estimates, because BLUP estimates will approach the overall mean of zero, if there is little data available for a reliable estimate. However, the sparse matrix in our data could not have influenced the observed groupings in MRT analysis because shrunk BLUPs will not account for much variance. In unconstrained cluster analysis, this could result in artificial groups of sites that are

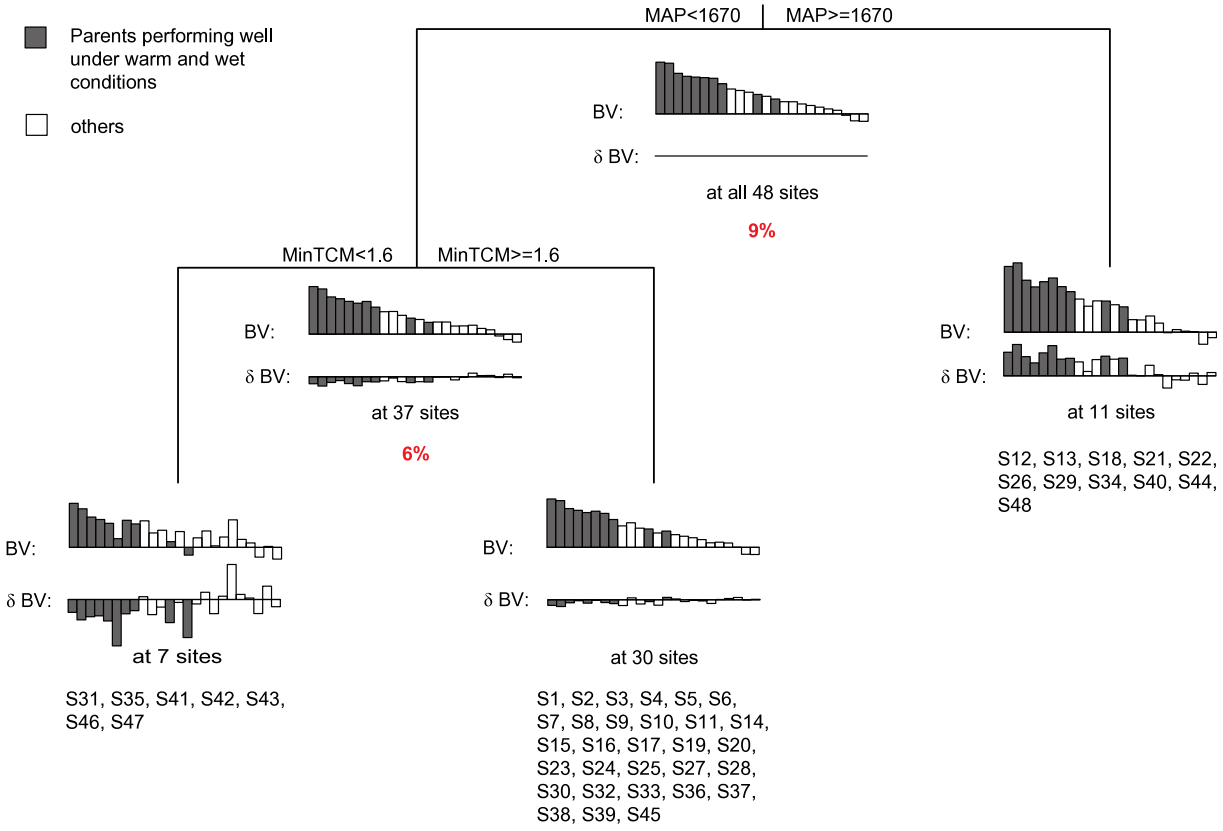

Fig. 3. Constrained cluster analysis, grouping 48 planting sites according to the performance of 24 parental genotypes, constrained by mean annual precipitation (MAP) and minimum temperature of the coldest month (MinTCM).when performance of 24 parental genotypes is constrained by mean annual precipitation (MAP) and minimum temperature of the coldest month (MinTCM). Genotypes are ordered from left to right by breeding value (BV), and the lower bar chart displays how genotypes deviation from the average breeding value at a particular group of sites (δBV). Variances in breeding values explained by each node of the cluster are indicated in red. Site codes are shown below each group.

similar because they all lack data (i.e. estimates are consistently zero), but such groups do not exist (Figs. 1a and 2). Since groups have to be defined by an external variable, such a problem cannot conceptually occur in the constrained analysis (Figs. 1b and 3).

4.2. Genetic gains by accounting for G × E at cold sites

That said, cross-over interactions that could be exploited by selecting genotypes for specific environments were detected in the second split of the constrained cluster analysis (Fig. 3). These sites of the left group, characterized by low minimum temperatures, are in Otago, Nelson and at high elevations in the Central North Island region. For deployment purposes, it appears possible to group the sites into warm, high-rainfall sites where the top-ranked RPBC parents perform consistently well, and cold sites where specific genotypes need to be selected for maximum performance. For example, we used the cut-off value of minimum temperature of the coldest month below 1.6 °C from constrained clustering to produce a map of suitable deployment areas, and it is apparent that a substantial portion of radiata pine plantations fall into this category (Fig. 4). A similar map (not presented) of the South Island showed that most of the Otago-Southlands region fell below the MinTCM of 1.6 degrees Celsius. It is also apparent that more trials are needed on high-elevation, cold sites on both the North and South Island. From a total of 48 trials, only seven fell into the low MinTCM category.

Fig. 4 also shows that a relatively large deployment area falls into this category that appears underrepresented by test sites. Of a total of 1,157,600 ha of plantation area on the North Island, and 396,500 ha of plantations on the South Island (Statistics New Zealand, 2011), approximately 16% and 74% fell into the MinTCM < 1.6 °C category, respectively. To illustrate potential gains from targeted deployment, we assume that we select 10 of 24 parents to include in a new production population (e.g., a new seed orchard, or in a crossing program to produce seed for vegetative propagation). Our reference group and reference genetic gain calculation is equivalent to the current practice of using a single breeding value for all sites. If we now introduce regional deployment population for the wet group (MAP > 1670 mm) represented by 11 sites in Fig. 3 and select the top 10 performers based on this bar chart, expected genetic gains would be 3% relative to the reference group. Optimizing deployment for the cold group (MinTCM < 1.6 °C) has a much larger effect. Selecting the top-10 parents from this group yields a 20% improvement in performance relative to the reference selection (top 10 overall performers). This suggests that it is feasible to increase plantation productivity and realize genetic gain through targeted deployment for the cold sites.

4.3. Limitations of environmental data

Minimum temperature has previously been identified as a potential driver of G × E in radiata pine in New Zealand, in addition to extreme maximum temperatures (McDonald, 2009). Likewise, cold high-elevation sites were found to be responsible for G × E of radiata pine in Australia (Wu and Matheson, 2005; Raymond, 2011; Gapare et al., 2012). It should be noted that drivers of G × E do not necessarily imply that these environmental variables are generally responsible for limiting productivity. A study by Watt et al. (2010) reported that variables driving productivity in New Zealand included mean annual temperature, available root zone

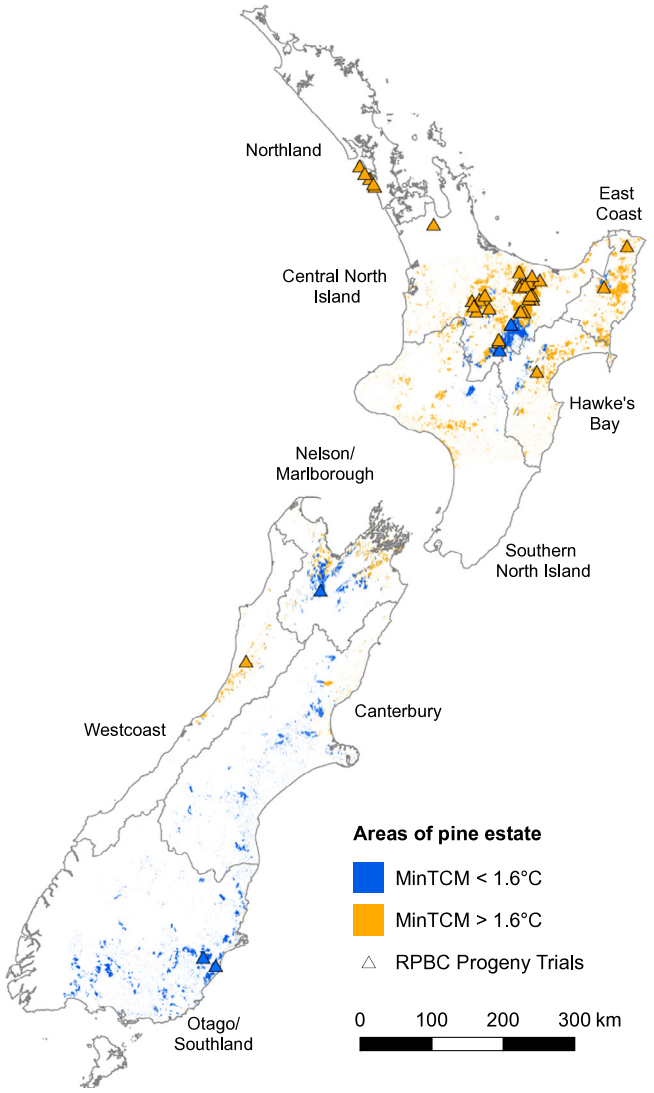

Fig. 4. Map showing radiata pine progeny trial sites (triangles) and plantation areas (colored), with minimum temperature coldest month (MinTCM) < 1.6 °C, where a high degree of G × E can be expected, and >1.6 °C, where minimal G × E can be expected (according to Fig. 3 left group). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

water storage, mean annual wind speed, length and slope factor and major soil parent material. However, in our case, we specifically screen for interactions, not absolute effects on productivity, which were removed by normalizing the data for each test site.

The second major limitation of this analysis relates to the accuracy of environmental data. This limitation is highlighted by the discrepancies between the unconstrained and constrained cluster analyses. The most salient example is the first split of the unconstrained provenance trial analysis (Fig. 1a), which is simply missing in the constrained analysis, while the subsequent groups are similar. If it was of practical importance to target this particular site type represented by the Kaniere and Waimihia sites, one would have to identify what particular soil or climatic characteristics that set these sites apart. Furthermore, if the responsible environmental variable was identified and verified, one would need to be able to map the driver of G × E in order to guide general reforestation. Thus, practical applications are limited to identifying the drivers of G × E, but also to accurately mapping these variables so that selected genotypes could be deployed to the appropriate environments.

The third important issue is collinearity among predictor variables. Correlation does not imply causation, and the true driver of G × E could be an environmental variable that we did not measure (or that we did not measure accurately), but that is collinear with another variable that was available for the analysis. To give a hypothetical example from the progeny trial analysis, consider the first split in Figs. 2 and 3. Fig. 3 indicates that mean annual precipitation is responsible for this split, but it is probable that the true underlying driver is water availability to the plant, which is also influenced by soil and topographic factors. Precipitation is only one aspect of water availability and could therefore only explain a relatively small fraction of the variance of the unconstrained cluster analysis.

In summary, results from any correlative analysis need to be interpreted carefully, and multivariate regression trees are no exception. From a practitioner's perspective, there appears to be sufficient evidence from this analysis as well as prior research that minimum temperatures (or otherwise indicated as high-elevation sites) are a significant source of G × E in radiata pine (e.g., McDonald, 2009; Raymond, 2011). This should enable us to make better-informed decisions on how genotypes may be allocated to cold sites in order to maximize productivity.

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References

Beeck, C., Cowling, W.A., Smith, A.B., Cullis, B.R., 2010. Analysis of yield and oil from a series of canola breeding trials. Part I. Fitting factor analytic mixed models with pedigree information. Genome 53, 992–1001.

Burdon, R.D., 1977. Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. Silv. Genet. 26, 168–175.

Burdon, R.D., Bannister, M.H., Low, C.A., 1992. Genetic survey of *Pinus radiata*. 2: Population comparisons for growth rate, disease resistance, and morphology. NZ J. For. Sci. 22, 138–159.

Burdon, R.D., Firth, A., Low, C.B., Miller, M.A., 1997. Native provenances of *Pinus radiata* in New Zealand: performance and potential. NZ J. For. Sci. 41 (4), 32–36.

Butler, D.G., Cullis, B.R., Gilmour, A.R., Gogel, B.J., 2009. ASReml-R reference manual, release 3. Technical Report. NSW Department of Primary Industries.

Carson, S.D., 1991. Genotype x environment interaction and optimal number of progeny test sites for improving *Pinus radiata* in New Zealand. NZ J. For. Sci. 21, 32–49.

Chen, L., Mi, X., Comita, L.S., Zhang, L., Ren, H., Ma, K., 2010. Community-level consequences of density dependence and habitat association in a subtropical broad-leaved forest. Ecol. Lett. 13, 695–704.

Costa e Silva, J., Dutkowski, G.W., Gilmour, A.R., 2001. Analysis of early tree height in forest genetic trials is enhanced by including a spatially correlated residual. Can. J. For. Res. 31, 1887–1893.

Costa e Silva, J., Potts, B.M., Dutkowski, G.R., 2006. Genotype by environment interaction for growth of Eucalyptus globulus in Australia. Tree Genet. Genomes 2, 61–75.

Cullis, B.R., Jefferson, P.A., 2012. Statistical approach for breeding value estimation in large multi-environment trials. School of Mathematics and Applied Statistics Technical Report Series, University of Wollongong, 27 pp.

Cullis, B.R., Smith, A.B., Beck, C., Cowling, W.A., 2010. Analysis of yield and oil from a series of canola breeding trials. Part II: exploring GxE using factor analysis. Genome 53, 1002–1016.

Cullis, B.R., Jefferson, P., Thompson, R., Smith, A.B., 2014. Factor analytic and reduced animal models for the investigation of additive genotype-by-environment interaction in outcrossing plant species with application to a Pinus radiata breeding programme. Theory Appl. Genet. 127, 2193–2210.
De'Ath, G., 2002. Multivariate regression trees: a new technique for modeling species-environment relationships. Ecology 83, 1105–1117.
Dutkowski, G.W., Costa e Silva, J., Gilmour, A.R., Lopez, G.A., 2002. Spatial analysis methods for forest genetic trials. Can. J. For. Res. 32, 2201–2214.
Dutkowski, G.W., Wellendorf, H., Aguiar, A., Silva, J.C., Gilmour, A.R., 2006. Spatial analysis enhances modelling of a wide variety of traits in forest genetic trials. Can. J. For. Res. 36, 1851–1870.
Eldridge, K.G., 1978. Refreshing the genetic resources of radiata pine plantations. In: Division of Forest Research: Genetics Section Report Number 7 CSIRO. pp. 1–120.
Gapare, W.J., Baltunis, B.S., Ivkovich, M., Low, C.B., Jefferson, P., Wu, H.X., 2011. Performance differences among ex situ native-provenance collections of *Pinus radiata* D. Don. 1: potential for infusion into breeding populations in Australia and New Zealand. Tree Genet. Genomes 7, 409–419.
Gapare, W.J., Ivković, M., Dutkowski, G.W., Spencer, D.J., Buxton, P., Wu, H.X., 2012. Genetic parameters and provenance variation of *Pinus radiata* D. Don. 'Eldridge collection' in Australia 1: growth and form traits. Tree Genet. Genomes 8, 391–407.
Gauch, H.G., 1992. Statistical Analysis of Regional Yield Trials: AMMI Analysis of Factorial Designs. Elsevier, Netherlands, Amsterdam.
Gilmour, A.R., Gogel, B.J., Cullis, B.R., Thompson, R., 2009. ASReml User Guide Release 3.0. VSN International Ltd., Hemel Hempstead, HP1 1ES, UK. 372 pp.
Hamann, A., Gylander, T., Chen, P., 2011. Developing seed zones and transfer guidelines with multivariate regression trees. Tree Genet. Genomes 7, 399–408.
Hardner, C., Dieters, M., DeLacy, I., Neal, J., Fletcher, S., Dale, G., Basford, K., 2011. Identifying deployment zones for *Eucalyptus camaldulensis* x *E. globulus* and x *E. grandis* hybrids using factor analytic modelling of genotype by environment interaction. Aust. For. 74, 30–35.
Johnson, G.R., Burdon, R.D., 1990. Family-site interaction in *Pinus radiata*: implications for progeny testing strategy and regionalized breeding in New Zealand. Silvae Genet. 39, 55–62.
Kang, M.S., 2002. Genotype–environment interaction: progress and prospects. In: Kang, M.S. (Ed.), Quantitative Genetics, Genomics and Plant Breeding. CABI, Cambridge, pp. 221–243.
Larsen, D.R., Speckman, P.L., 2004. Multivariate regression trees for analysis of abundance data. Biometrics 60, 543–549.
McDonald, T.M., 2009. Making sense of genotype × environment interaction of Pinus radiata in New Zealand. Unpublished MSc. thesis, School of Forestry, University of Canterbury, Christchurch, New Zealand, 82pp.
Mead, D.J., 2013. Sustainable management of Pinus radiata plantations. FAO Forestry Paper No. 170. FAO, Rome, 257pp.
R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rais, A., van der Kuilen, Jan-Willem G., Pretzsch, H., 2014. Growth reaction patterns of tree height, diameter, and volume of Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) under acute drought stress in Southern Germany. Eur. J. Forest Res. http://dx.doi.org/10.1007/s10342-014-0821-7.
Raymond, C.A., 2011. Genotype by environment interactions for *Pinus radiata* in New South Wales. Tree Genet. Genomes 7, 819–833.
Raymond, C.A., Henson, M., 2009. Genetic variation within the native provenances of *Pinus radiata* D. Don. 1. Growth and form to age 26 years. Silvae Genet. 58, 242–252.
Sheaves, M., Abrantes, K., Johnston, R., 2007. Nursery ground value of an endangered wetland to juvenile shrimps. Wetlands Ecol. Manage. 15, 311–327.
Smith, A., Cullis, B.R., Thompson, R., 2001. Analyzing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. Biometrics 57, 1138–1147.
Statistics New Zealand, 2011. 2011 Agricultural Production Survey. <http://www.stats.govt.nz>.
Tait, A., Henderson, R., Turner, R., Zheng, X., 2006. Thin-plate smoothing spline interpolation of daily rainfall for New Zealand using a climatological rainfall surface. Int. J. Climatol. 26, 2097–2115.
Ward Jr., J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58, 236–244.
Watt, M.S., Palmer, D.J., Kimberley, M.O., Hock, B.K., Payn, T.W., Lowe, D.J., 2010. Development of models to predict *Pinus radiata* productivity throughout New Zealand. Can. J. For. Res. 40, 488–499.
Wilde, R.H., Willoughby, E.J., Hewitt, A.E., 2000. Data Manual for the National Soils Database Spatial Extension. Landcare Research New Zealand Ltd..
Wu, H.X., Matheson, A.C., 2005. Genotype by environment interaction in an Australia-wide radiata pine diallel mating experiment: Implications for regionalized breeding. For. Sci. 5, 1–11.
Wu, H.X., Ying, C.C., 2004. Geographic patterns of local optimality in natural populations of lodgepole pine. For. Ecol. Manag. 194, 177–198.