Breeding for cross resistance in needle retention in *Pinus radiata*

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Summary

Cyclaneusma minus and *Dothistroma septosporum* (Dorog.) M. Morelet*,* are both pathogens that cause needle diseases affecting *Pinus radiata* D. Don in New Zealand. These needle diseases are routinely assessed in breeding trials, however assessment of each disease is often confounded by the presence of the other. This study was aimed at determining the genetic correlation between Dothistroma needle blight and Cyclaneusma needle cast resistance where there was no observable confounding. Genetic correlation found between traits was favourable and strong (-0.78) indicating that selection for higher resistance to needle loss caused by *Dothistroma* infection will improve the resistance to needle loss caused by *Cyclaneusma* infection.

Keywords: needle retention, heritability, genetic correlation, disease resistance

Introduction

Dothistroma needle blight was first observed in New Zealand in 1962 (Bulman et al., 2004) and Cyclaneusma needle cast was first documented in the 1950s (Bulman and Gadgil, 2001). Dothistroma needle blight occurs in young trees up to age 15, and defoliation is most apparent in spring. Cyclaneusma needle cast can infect trees older than approximately three years, but is most prevalent on trees aged 11 to 20 years (van der Pas et al., 1984a). Infection is prevalent in spring and occasionally late autumn with cast needles being yellow brown in colour. Both diseases are widespread in warm moist conditions found within the Central North Island of New Zealand (Watt et al., 2011b; Watt et al., 2012).

van der Pas et al. (1984b) evaluated the impact of Cyclaneusma on the growth of *Pinus radiata* in New Zealand and found that a 10% increase in the proportion of diseased trees equated to a loss in timber volume of 10-14 m^3 ha^{-1}. Estimated loss in volume due to Dothistroma needle blight, of 7-13 m^3 ha^{-1} per 10% increase in the proportion of diseased trees, was similar (van der Pas, 1981). To put these values into context, the cost of Dothistroma needle blight to the New Zealand forest industry has been estimated at $19.8 million (NZD) per annum due to lost volume and the cost of control by aerial spraying copper oxides (Watt et al., 2011a). whereas forestry contributes an annual gross income of around $ 5 billion (NZD) (FOA, 2015). The cost of Cyclaneusma needle cast is likely even higher being estimated at $38 million (NZD) per annum for trees aged between 6 and 20 years (L. Bulman, unpublished report 2009).

Wilcox (1982) estimated heritability of ~0.4 for Dothistroma needle blight resistance, which would enable effective selection of resistant families and/or individuals at age 5 or younger. Heritability estimates for needle retention due to

Cyclaneusma needle cast ranged between 0.40 and 0.47 (Kumar et al., 2008). However, selection forresistance was less straightforward, with the disease expression occurring at a later age (~ age 8) making it harder assess compared with Dothistroma needle blight due to the height of tree crowns at this age. One possible solution to facilitate the selection for Cyclaneusma needle cast is using the multiple trait models to estimate the genetic correlation between Dothistroma and Cyclaneusma and use the genetic correlation to perform selection for Cyclaneusma based on Dothistroma by generating the correlated response, which lead to simplification of selection for disease resistance in the current breeding programme for radiata pine. The objective of this study was therefore to see if this was possible by estimation of the genetic correlation between the needle damage from *Dothistroma* and from *Cyclaneusma* in New Zealand radiata pine.

Materials and methods

Three progeny trials from the Radiata Pine Breeding Company (RPBC), New Zealand breeding population, were used to estimate the genetic parameters for disease resistances in the current study (Table 1). Two of the trials were tested using incomplete block design with single tree plots planted in 2014 across two sites, Kaingaroa and Kinleith forests, located in the North Island of New Zealand (Table 1). The total number of trees assessed at both sites were 5,781 trees, assessed for needle cast caused by Dothistroma needle blight infection when trees were two and three years from planting.

The third trial located in Kinleith forest (Table 1), and was assessed for Cyclaneusma needle cast. This trial did not suffer from infection by Dothistroma needle blight. Trial design was an incomplete block design with single tree plots planted in 2008. This trial contained 4,195 trees assessed for Cyclaneusma needle cast when the trees were 8 years of age from planting.

Traits definition

The traits reflecting needle disease resistance were evaluated as follows:
1) Needle blight due to Dothistroma needle blight was scored as percentage of crown affected, at 5 percent intervals from 0 to 90% (meaning that a high score reflects low resistance)
2) Needle retention to describe resistance to Cyclaneusma needle cast was scored as:
0: No needles retained; 1: Partial retention of 1-year-old needles; 2: Only 1-year-old needles retained; 3: All 1-year-old needles retained, and partial retention of 2-year-old needles; 4: All 1- and 2-year-old needles retained; 5: All 1 and 2-year-old needles retained and partial retention of 3 year-old needles; 6: All 1, 2 and 3-year-old needles retained (meaning that a high score reflects high resistance).

The pedigree was traced back as many generations as possible in the forest genetics database (Scion Rotorua, New Zealand). The pedigree file included 10,987 trees.

Statistical analysis

The mixed procedure in the SAS package (ver. 9.4; SAS Institute Inc., Cary, NC) was used to test for fixed effects in the model. Genetic analysis was performed with the average information REML algorithm in ASReml package in R environment (Butler et al., 2009). Bivariate analysis was performed to estimate genetic correlations between the traits. The following model in scalar notation was used for the genetic analysis of Dothistroma needle blight damage in a bivariate model:

$$y_{ijk} = \mu + a_i + bs_j + n_k + e_{ijk} \qquad (1)$$

where y_{ijk} is the observation of the trait Dothistroma needle blight damage; μ is the overall mean; a_i is the random genetic effect which was assumed to be normally distributed with distribution ~N, where is the additive genetic variance and A is the additive genetic relationship matrix; b_{sj} is the random block by site interaction effect ~N, where is block by site interaction variance; n_k is non additive genetic effect of the individual genotype within the genetic material ~N, where is non additive genetic variance, and e_{ij} is the random residual which was assumed to be normally distributed with distribution ~N, where is the residual variance.

To analyze the needle retention after damage caused by Cyclaneusma needle cast, the following model was used

$$y_{ijkl} = \mu + icb_i + r_j + c_k + a_l + e_{ijkl} \qquad (2)$$

where y_{ijkl} is the observation of the trait needle retention after damage caused by Cyclaneusma needle cast; μ is the overall mean; icb_i is the fixed effect of incomplete block i ($i = 152$ incomplete blocks); r_j is the fixed effect of row j ($j = 156$ rows); c_k is the fixed effect of column k ($k = 48$ columns); a_l is the random genetic effect which was assumed to be normally distributed with distribution ~N, where is the additive genetic variance and A is the additive genetic relationship matrix , and e_{ijkl} is the random residual which was assumed to be normally distributed with distribution ~N, where is the residual variance.

The variance- covariance structure for the bivariate model is

$$Var \sim N$$

where a_1 and a_2 represent the additive genetic merit for the traits needle damage after either Dothistroma needle blight or needle retention after damage from Cyclaneusma needle cast, respectively; and are the additive genetic variances for the traits; and is the additive genetic covariances between traits. Because the correlated traits are not recorded on the same trees, no environmental covariances exists between traits, and the residual covariance matrix is

$$Var \sim N$$

Where I is the identity matrix; , are the residual variances for the traits: needle damage by Dothistroma needle blight or, needle retention after damage by Cyclaneusma needle cast, respectively.

Results and Discussion

Descriptive statistics

The mean of infection for needle damage due to Dothistroma needle blight was 25.2% of the crown whereas, the average needle retention score after Cyclaneusma needle cast was 2.55.

Genetic correlation

Genetic correlation between needle damage due to Dothistroma needle blight and needle retention after damage from Cyclaneusma needle cast was negative (which is favourable) and strong (0.78±0.11), indicating that genotypes with low Dothistroma needle blight infection score (which is considered highly resistant) would also have high score for needle retention due to resistance to Cyclaneusma needle cast.

These results have important applications. First, selection for Dothistroma needle blight resistance will increase resistance to Cyclaneusma needle cast, this strong genetic correlation allows early selection for Cyclaneusma needle cast based on Dothistroma needle blight score, which would have a significant impact on decreasing the cost and logistics required for assessment, especially for Cyclaneusma needle cast. Additionally, selection for resistant genotypes would enable the forest growers to grow the dual resistance genotypes in the areas where both diseases are prevalent.

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Table1. Description of progeny trials at Kaingaroa, Kinleith, forests in New Zealand

Trial	Kaingaroa forest	Kinleith forest	_Kinleith forest
Disease assessment	Dothistroma		Cyclaneusma
Planting year	2014	2014	2008
Latitude	38° 24' 30.582"	38 ° 18' 52.7"	38° 15' 56"
Longitude	176° 33' 52.6428"	176° 00' 51.2"	176° 04' 30"
No. planted trees	2,896	2,885	4,195
No. families	63 open pollinated	63 open pollinated	172 open pollinated
Trial design	Incomplete block, single tree plot	Incomplete block, single tree plot	Incomplete block, single tree plot