GENOTYPIC VARIATION IN SYMPTOMS OF UPPER MID-CROWN YELLOWING AND *CYCLANEUSMA MINUS* IN A *PINUS RADIATA* STAND

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(Received for publication 11 July 1996; revision 28 July 1997)

ABSTRACT

In New Zealand, low needle retention in *Pinus radiata* D.Don is often associated with infection by a needle-cast fungus, *Cyclaneusma minus* (Butin) DiCosmo *et al.*, and with a condition known as upper mid-crown yellowing (UMCY). Variability in the expression of these two disorders is known to be controlled by both environmental and genetic factors. In order to estimate the degree of genetic control, adjacent seedlings and clonal trees (six clones selected for vigour in the nursery) were assessed at age 22 years for symptoms of UMCY and of *C. minus* in stands thinned to three final-crop stocking rates. Estimates of genotypic and non-genetic variance and of broad-sense heritability (ratio of genotypic to phenotypic variance) were obtained in two ways: (a) by subtracting the observed within-clone variance from the phenotypic variance of seedling trees, and (b) by directly estimating clone-to-clone variance. Values obtained by these methods agreed well with each other and with earlier estimates made in a nearby seed orchard. Factors related to method of propagation and physiological ageing are therefore unlikely to inflate clonal variation. Clones may be the more cost-effective indicator for UMCY studies. Genotypic variance in resistance to both disorders is considered to be sufficiently high (at least 64% for UMCY and 44% for *C. minus*) to warrant consideration in silvicultural practices.

Keywords: upper mid-crown yellowing; needle-cast; genetics; clonal forestry; heritability; tree health; *Cyclaneusma minus*.

INTRODUCTION

Pinus radiata stands in New Zealand can exhibit crown thinning and dieback due to a nutritional disorder known as upper mid-crown yellowing (UMCY) (Beets *et al.* 1993). In addition, low needle-retention can be caused by the needle-cast fungus *Cyclaneusma minus* (Gadgil 1984). Between-tree variation in terms of both UMCY and *C. minus* symptoms is large, and complicates field-based assessments of growth loss. Research has therefore focused on procedures for distinguishing between these disorders, and on methods of scoring the severity of crown loss (Beets & Jokela 1994), as prerequisites for ameliorating these conditions. Use of this approach has avoided some of the difficulties encountered by European researchers studying dieback phenomena (Kandler 1992).

Clones of *P. radiata* are known to differ in their expression of UMCY and C. *minus* symptoms (Beets & Jokela 1994), with broad-sense heritability estimated to be 59% and 68%, respectively. Beets & Jokela (1994) were unable to ascertain whether estimation of the variation in UMCY symptoms in seed-orchard clones over-emphasised the genotypic component, as there was a possibility that clonal variation estimates might be inflated by propagation practices and/or physiological age (*see* Burdon & Shelbourne 1974). Despite the uncertainty about using the variance among clones to estimate heritability, it was expected that within-clone variation in UMCY would be a reliable indicator of variation attributable to environmental factors.

The heritability of traits such as UMCY and needle retention can also be estimated in stands where seedling and clonal *P. radiata* are growing in mixture. Here phenotypic variation in UMCY and needle retention characteristics can be partitioned into environmental (microsite in this situation) and genetic components, without the need to calculate the variance among clones. This approach allows a comparison between clonal differences and genetic differences.

The objectives of the study reported here were (i) to examine the effects of clone and microsite on the mean severity of UMCY and *C. minus* symptoms in *P. radiata*, and (ii) to compare variability in UMCY and needle retention in clonal material with that in non-clonal material.

MATERIALS AND METHODS

Site Description and Experimental Material

The trial was located at Puruki Forest, which is located in the Purukohukohu Experimental Basin in the central North Island. A major portion of New Zealand's exotic forests have been planted on the volcanic soils of this region. Puruki is an ex-pasture site which was planted with *P. radiata* in 1973. Stem volume increment has exceeded 50 m^3/ha annually in closed-canopy stands (Beets & Brownlie 1987). Previous land-use, soil, climate, and management of this first-rotation plantation have been described in detail by Beets & Brownlie (1987).

To summarise, after uniform spraying with herbicide to kill pasture plants, the 35-ha catchment was planted in 1973 with 1-year-old climbing-select (a largely unimproved standard used by the FRI) *P. radiata* seedlings (2200 trees/ha; 2.4 × 1.8 m spacing). In three blocks totalling 1.2 ha, every third seedling in every third row was replaced by a rooted cutting of one of six clones (FRI Clone No. 448, 450, 451, 454, 455, 456) in an otherwise fully random layout (Beets & Kimberley 1993). Different thinning regimes were later applied to these blocks.

The three blocks had been sited for maximum comparability in terms of aspect and slope. One assessment plot (0.33, 0.27, 0.40 ha in area) was installed in each block at 60, 180, and 550 stems/ha final-stocking rates, respectively. The topography of the plot with 550 trees/ha was more undulating than the other two plots which had a predominantly south-easterly aspect. The trees were pruned to 2.2 m height and final-crop stockings were achieved by repeated thinning (Beets & Kimberley 1993). The final thinning to 550 trees/ha was undertaken at age 7 years, to 180 trees/ha at age 15 years, and to 60 trees/ha at age 14. The on-site selection ratios for seedling material were therefore 1:4, 1:12, 1:36. To meet the

objectives of this study, the assessment plots at Puruki needed to be small, to ensure as far as possible that tree-to-tree variability in UMCY and needle retention symptom expression was related to microsite variation or genetic make up.

The six clones had been propagated from the most vigorously growing 6-month-old seedlings in open-sown nursery beds in 1964. They were subsequently maintained for experimental use as clonal hedges (Knight 1978; Jackson *et al.* 1973). The chronological age of the rooted cuttings in 1973 was 9 years, and then physiological age as cuttings taken from hedges was about 5 years.

Tree Health Scores

The severity of UMCY symptoms and the needle loss resulting from *C. minus* were scored in October 1995 at stand age 22 years, following the methods of Beets & Jokela (1994). Using their scoring system, individual tree UMCY scores can range from 1 (no symptoms) to 8 (severe upper crown needle loss and crown dieback), and needle retention scores can range from 0 (no retention) to 3 (3 or more years' needle retention in the lower crown).

Trees growing at 60 and 180 trees/ha were scored with the aid of binoculars by two observers positioned on the ground. Two scorers were used to reduce the chance of errors arising from viewing difficulties. Scorers were required to agree on the score assigned to each tree. Ground-based scoring for UMCY is not usually possible at high stocking rates, because the upper crowns can be obscured from view by neighbouring trees. Fortuitously, a meteorological mast had been installed in the plot at 550 trees/ha. One observer was positioned above the canopy on top of this meteorological mast to score UMCY in these plot trees. Trees with severe *C. minus* symptoms (approximately 10% of the trees) were not scored for UMCY, because UMCY symptoms were masked. To maintain comparability of micro-site conditions only seedling trees growing adjacent to a clone were scored. This restriction would allow sufficient seedling-trees to be scored for statistical purposes (a total of 73 seedling-trees and 57 clonal-trees).

Calculations and Statistical Analyses

Data from clonal trees were analysed as for single-tree plots using ramets of a clone as replicates. Two-way analysis of variance was used to test for clone, stocking, and interaction effects (as fixed effects) on UMCY and needle retention at stand age 22 years. The stocking effect was considered to be a site effect when interpreting the following results (hence the tables are labelled with stocking, with site given in parentheses), because UMCY and needle retention varied spatially at Puruki independently of stocking.

The phenotypic (V_P) variance in UMCY and needle retention was partitioned into its two components (genotypic variance V_G and environmental variance V_E). For each stocking, V_P was estimated as the variance among seedling-origin trees, V_E as within-clone variance, and V_G as $V_P - V_E$ (Falconer 1960). Broad-sense heritability (H_{BS}) was estimated as V_G/V_P (Zobel & Talbert 1984), using statistics derived from both clonal and seedling material. Using another method, variance among clones (V_C) was expressed as a percentage of $V_C + V_E$. This purely clone-based estimate of broad-sense heritability was compared with the estimate of H_{BS} based on composite statistics.

RESULTS

UMCY and *C. minus* Symptom Scores

Means and error mean squares (s^2) for the two health traits in seedlings and clones are given in Table 1. For UMCY, the clonal data showed significant effects of clone ($p < 0.0001$) and clone × stocking (i.e., site) interaction ($p = 0.02$), but no significant main effect of stocking ($p = 0.1$). Among the clones, No. 448 and 456 were free of UMCY (mean score of 1.0 at all stockings). More severe UMCY evident at the lowest stocking in Clones 450 (mean score = 4.7) and 454 (mean score = 3.6) resulted in the significant clone × stocking interaction.

TABLE 1–Means and variances (s^2) of health scores of upper mid-crown yellowing (UMCY) and *C.minus* symptoms (needle retention) in 22-year-old seedling and clonal *P. radiata* at three stocking rates (trees/ha)

Stocking (site)	UMCY				Needle retention			
	Seedlings		Clones		Seedlings		Clones	
	Mean	s^2	Mean	s^2	Mean	s^2	Mean	s^2
60	3.2	3.36	3.0	0.66	1.0	0.67	0.6	0.29
180	2.3	2.72	1.7	0.28	0.8	0.48	0.6	0.17
550	2.2	2.19	1.9	0.41	1.6	0.56	1.2	0.39
Overall	2.78	2.68	2.29	0.56	1.07	0.53	0.79	0.30

This small set of six clones indicates a key point—that clone and seedling mean UMCY scores move in parallel from site to site (Table 1). Absolute values of the clone means is of little importance, given the small number of clones involved.

For needle retention, clone ($p < 0.0001$) and stocking ($p = 0.009$) effects were significant, but the interaction effect was not ($p = 0.2$). Clones 448, 450, and 454 were most susceptible. Needle retention was greatest in seedlings and clones growing at the highest stocking (Table 1). Only one clone (456) was tolerant/resistant to both *C. minus* and UMCY.

Variation of UMCY and *C. minus* symptoms was large in seedling material (Table 1). Using statistics from the overall analysis (where stocking and interaction effects were not included), it was found that approximately 35 and 45 seedling-trees would have to be scored for UMCY and needle retention respectively, to achieve stand estimates within 20% of the mean at the 95% confidence level. For UMCY, approximately 10 clonal trees would have to be scored to achieve comparable precision. Fewer individuals would be required with improved stratification. For example, only six individuals of Clone 454 would be required at the lowest stocking rate.

Source of Variation in UMCY

Phenotypic, genotypic, and environmental variance in UMCY tended to be greatest where UMCY symptoms were most severe (lowest stocked stand) and least where the severity of UMCY was lowest (highest stocked stand) (Table 2). The ratio of genotypic to phenotypic variance (i.e., broad-sense heritability based on composite statistics) was, therefore, not greatly affected by stocking (Table 2).

TABLE 2–Phenotypic coefficients of variation (CV_p) (%), components of variance in UMCY symptoms in *P. radiata*, and heritability estimates (V_G/V_P) at three stocking levels (trees/ha)

Stocking (site)	CV_P	V_P seedlings	V_E clones	V_G (V_P-V_E)	V_G/V_P		
					Composite method	Clone-only method	Beets & Jokela (1994)
60	57	3.36	0.66	2.70	0.80	0.71	
180	56	2.72	0.28	2.44	0.90	0.41	
550	66	2.19	0.41	1.78	0.81	0.26	
Overall	59	2.68	0.56	2.12	0.79	0.64	0.59

The pattern of variation of *C. minus* symptoms was complex (Table 3). Environmental variation was not obviously related to stocking rate, but was greatest in the plot at the highest tree density where, on average, defoliation was least severe. The topography was more undulating there than in the other two plots, where solar radiation on the general south-easterly aspect would be lower—which results in conditions which tend to favour *C. minus*.

Broad-sense heritabilities (H_{BS} estimated as V_G/V_P) (Tables 2 and 3), based on the composite and clone-only methods, were compared with the earlier estimates of Beets & Jokela (1994). For UMCY, the clone-only estimates of H_{BS} tended to be lower than those based on composite statistics. The statistical significance of H_{BS} estimates was obtained by testing the null hypothesis, $H_0 : V_P = V_E$. For UMCY, the overall $F_{65,43} = 2.677/0.562 = 4.76$ ($p < 0.001$) (Table 2). For needle retention, the overall $F_{69,48} = 0.534/0.301 = 1.77$ ($p < 0.05$) (Table 3). The heritabilities based on statistics obtained for stocking rates were rarely significant.

TABLE 3–Coefficient of variation, and components of variance of needle retention in *P. radiata*, and heritability estimates (V_G/V_P) at three stocking levels (trees/ha)

Stocking (site)	CV_P	V_P seedlings	V_E clones	V_G (V_P-V_E)	V_G/V_P		
					Composite method	Clone-only method	Beets & Jokela (1994)
60	82	0.67	0.29	0.38	0.56	0.31	
180	85	0.48	0.17	0.31	0.64	0.77	
550	48	0.56	0.39	0.17	0.30	0.40	
Overall	68	0.53	0.30	0.23	0.44	0.54	0.68

DISCUSSION

Stocking and other environmental effects were confounded in the statistical analysis, because of the lack of true replication. While there was a trend towards increasing UMCY severity with decreasing stocking rate, other (unpublished) studies at Puruki have noted large differences in mean UMCY severity within uniformly stocked stands and these have been attributed to soil properties. It would therefore be wise to ignore the stocking effects observed here.

Variances among seedlings and within clones gave a higher estimate of the broad-sense heritability of UMCY (79%) than was obtained using the variance between clones, both in this study (64%) and in a study of 86 seed orchard clones (59%) (Beets & Jokela 1994). At comparable plot mean UMCY values, V_E at Puruki would have to exceed that found at the seed orchard in order to reduce Puruki V_G/V_P values to below 59%. This is unlikely given the small plot sizes at Puruki. We therefore conclude that clonal differences in UMCY were not inflated by physiological ageing.

The estimated broad-sense heritability of *C. minus* symptoms was 44% using composite statistics, compared to 54% at Puruki and 68% at the seed orchard using the clone-only method (Beets & Jokela 1994). Similar values for broad-sense heritability (42%) were reported by Burdon *et al.* (1992b). Narrow-sense heritabilities for crown retention have been estimated to be approximately 25–30% (Burdon 1992; Burdon *et al.* 1992a). The estimated narrow-sense heritability and economic importance of resistance to this disease explain why it has qualified for inclusion in the tree breeding programme at FRI (Burdon 1992).

For research purposes, closer attention to the mapping of environmental conditions favouring *C. minus* and UMCY is warranted. Mild, wet, climatic conditions favour Cyclaneusma needle-cast (Gadgil 1984), which is more severe on southerly aspects and gully sites. The broad-sense heritabilities of UMCY and needle retention observed in this study indicate that environmental variation within plots can be low over areas of at least 0.25 ha. Prior mapping of sites, coupled with the use of indicator clones to overcome variability due to genetic noise, will assist in development of cost-effective trial designs for investigating *C. minus* and UMCY, and lead to improved methods for operationally matching clones to sites.

Management of *C. minus* by delaying final thinning until differential resistance to *C. minus* is expressed (age 6 years) increases stand growth after the removal of susceptible genotypes (L.Bulman pers. comm.). It was assumed that no improvement in tolerance to *C. minus* and UMCY through selective thinning occurred at Puruki, even though the selection criteria for thinning favoured trees with healthy crowns (Beets & Kimberley 1993). This assumption is likely to be valid for UMCY, given the difficulties experienced in observing UMCY from the ground. Operational selection of tolerant genotypes will not be possible until forestry workers are trained to recognise the symptoms.

Similarly, current forest practice does not utilise tolerant genotypes to reduce the severity of UMCY, which is difficult to recognise and assess in normal forests and in progeny tests. The case for including tolerance to UMCY as a tree breeding goal will require confirmation of the narrow-sense heritability of UMCY and its economic importance. Clonal forestry offers a direct method for securing rapid gains in tolerance to UMCY on sites prone to that disorder.

ACKNOWLEDGMENTS

We thank Trevor Faulds for propagation of the clonal material, and Dr D.S.Jackson for his involvement in establishing the Puruki trial site, and in particular for the provision of clones.

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