GENETIC VARIATION AND INHERITANCE OF RESISTANCE TO DOTHISTROMA NEEDLE BLIGHT IN PINUS RADIATA

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(Received for publication 2 June 1982)

ABSTRACT

Three separate studies showed that New Zealand populations of **Pinus radiata** D. Don possess useful quantitative genetic variation in resistance to infection by the fungus **Dothistroma pini** Hulbary. Heritability of resistance was high enough under suitable conditions to allow effective selection and breeding for resistance.

In the first study, 66 "resistant" trees were phenotypically selected in heavily diseased New Zealand plantations in 1966; 21 of these subsequently retained a strong measure of resistance when clonally propagated by cuttings. However, only five clones were judged acceptable in growth, branching, and stem straightness.

In the second study, clonal variation in resistance to natural infection by the fungus was sharply exhibited in hedged clonal archives from 2- and 3-year-old second-generation ortets (originally selected for growth and stem quality, but not for resistance). Although closely related hedged clones sometimes differed considerably in their susceptibility to infection, there were still significant mean differences in the susceptibility of families, attributable almost entirely to general combining ability effects of the parents. Variation in susceptibility was also strong among hedges of clones aged 18–23 years.

The third study showed dramatically that there were marked genetic differences among 5-year-old trees in resistance to infection under natural inoculation in the field. The study involved a diallel cross among 25 seed orchard clones. General combining ability effects were very strong, while specific combining ability effects were negligible. Individual full-sib families ranged from 3% to 69% in proportion of progeny badly infected.

The strong additive genetic variation and potentially high heritability in resistance within current breeding populations of **P. radiata**, already improved in growth and stem quality by intensive selection and breeding, augurs well for the successful incorporation of needle blight resistance into future breeds via traditional general-combining-ability seed orchards.

INTRODUCTION

The needle blight disease caused by the fungus *Dothistroma pini** (syn. *Dothistroma septospora* (Dorog.) Morelet) is the most serious threat yet to the health of New

* **Dothistroma pini** is the imperfect stage of **Scirrhia pini** Funk & Parker.

Zealand's forests of *P. radiata.* It could also become important in Australia (Edwards & Walker 1978). Heavy infections can cause severe defoliation to young stands over large areas, thereby slowing down the growth of the trees. If left unchecked in areas of high disease hazard, infection can kill young trees, as has commonly occurred in Kenya (Allen 1973; Gibson 1972, 1979).

Since 1966, outbreaks of the disease have been countered by aerial spraying with copper fungicide (Kershaw *et al.* 1979). Chemical spraying is fully accepted in New Zealand as a worthwhile immediate control measure against Dothistroma needle blight but it is not always effective in preventing re-infection and increment loss, and is becoming more expensive because of rising costs and the ever-increasing area of susceptible trees (60 000 ha sprayed in 1979 and 105 000 ha in 1980).

The severity of needle blight attack fluctuates from year to year according to weather conditions; wet summers promote the heaviest attacks. The disease is now obviously permanent in New Zealand, but strains of resistant *P. radiata* are unlikely to evolve here by natural selection as long as spraying continues. Artificial selection and breeding for resistance offers an alternative or complement to spraying as a method of reducing the disease hazard, provided that exceptionally virulent mutant forms of *D. pini* do not subsequently appear. Breeding for resistance in *P. radiata* could be accomplished in several ways:

- Hybridisation with resistant, other closed-clone pines, such as *Pinus patula* Schiede & Deppe;
- Selection of the most resistant provenances (Burdon & Bannister 1973; Cobb & Libby 1968);
- Selection and crossing of resistant parent trees from local stands or breeding populations.

In an investigation into the third of these possibilities, emphasis was given to establishing whether or not genetic variation in Dothistroma needle blight susceptibility within New Zealand *P. radiata* is great enough and sufficiently heritable to allow breeding of resistant strains. To be economically significant, resistance must effectively defend the crop against the disease. It could be sufficient to avoid having to spray at all, or at least so often. Alternatively, "effective" resistance combined with other silvicultural devices such as using cuttings instead of seedlings, early pruning, and heavy thinning, could help maximise the benefits from spraying in reducing inoculum load and minimising increment loss.

PHENOTYPIC SELECTION FOR RESISTANCE

There is obvious phenotypic tree-to-tree variation in Dothistroma needle blight resistance in any infected stand. A strong environmental component in this variability is often apparent, most noticeably in the localised severity of defoliation of trees growing in damp gullies, and other "hot spots" resulting from early spread from initial infection centres. Within areas of heavy infection, however, it is possible to find individual trees that show "field" resistance; these are likely to be either chance escapes or genetically resistant trees.

During October and November 1966, 79 apparently resistant trees were selected from 7- to 15-year-old heavily diseased, genetically unimproved *P. radiata* stands at Kaingaroa and Tokoroa that had never been sprayed with copper fungicides (Forest Research Institute 1968). About 220 ha of uniformly heavily infected and defoliated stands were systematically searched for healthy green trees; no account was taken of stem form, branching characteristics or growth rate. The stands were mostly naturally regenerated second crops which had been thinned and some of them had been pruned. All were growing on volcanic ash soils.

The trees that were still noticeably free of infection compared with their neighbours by May 1967 were retained as "resistant" plus-trees ("867" series*). These 66 selected "867" series clones were established in clonal tests of rooted cuttings at Rotorua and in Kaingaroa Forest in July 1968. The tests also included several clones not selected for needle blight resistance, from ortets aged 9–40 years, and some seedlings of a routine seedlot. Artificial inoculation using overhead frames covered with infected pine needles was employed in the Rotorua test, while natural infection was relied on in the two Kaingaroa tests, which were located inside heavily diseased stands in Compartments 44 and 913.

After 18 months the cuttings were clearly more resistant than seedlings (Table 1). There were no very marked differences in resistance between cuttings from ortets of different ages (cf. Garcia & Kummerow 1970), or between clones selected for resistance and unselected clones. There were, however, statistically significant differences between clones within both the selected and unselected groups.

TABLE 1—Mean defoliation 18 months after planting

	Defoliation (%)
Seedlings	49
Cuttings	
ex 40-year-old trees	18
ex 20-year-old trees	16
ex 15-year-old trees	17
ex 12-year-old trees	15
ex 9-year-old trees	11
Clones of 7- to 15-year-old "resistant" trees	12
Over-all mean	15

Although the mean defoliation levels of the selected (12%) and unselected (15.4%) clones were fairly similar, there were other differences between the two groups which suggested that phenotypic selection for resistance to infection was partially successful

* Trees incorporated in the **P. radiata** breeding programme are identified by the place where the trial is set up and the year in which they were selected, e.g., 8 = FRI, 67 = 1967.

and therefore that resistance must be heritable. For example, 48% of the selected clones were "resistant" (suffering less than 10% mean defoliation) as against 21% of the unselected clones. Alternatively, there were fewer "susceptible" clones (suffering 20% or greater mean defoliation) in the selected group (7%) than in the unselected group (29%).

In all, 21 clones of the "867" series were judged to be "resistant" by the criterion of less than 10% defoliation in clonal tests. These 21 clones were assessed in unhedged clonal archives at Rotorua in March 1981 to determine which ones, if any, were up to seed orchard standard with respect to growth, stem straightness, and branch quality. Only five (867–44, 53, 65, 75, 79), all originating from N.Z. Forest Products Ltd's forests at Tokoroa, were acceptable.

CLONAL VARIATION IN DISEASE RESISTANCE

Dramatic clonal variation in defoliation due to *D. pini* showed up unexpectedly in 1980 in archives maintained at Rotorua and Kaingaroa Forest. The pedigree of some clones was known so it was possible to analyse the variation to give information about mode of inheritance of disease resistance.

Hedges of Second-generation Clones at Rotorua

A series of 47 controlled-pollinated full-sib families involving 16 selected parents of the "850" series (current seed orchard clones) was planted in blocks in Cpt 1350 of Kaingaroa Forest in 1968. At ages 2, 3, and 5 years from planting, second-generation plus-trees were selected from within the families, on the standard selection criteria of fast growth, freedom from malformation, multinodal branching habit, and good stem straightness. Fifty trees were selected in 1970 ("870" series), 150 in 1971 ("871" series), and 160 in 1973 ("873" series). Each ortet of the "870" series, 101 of the "871" series, and 84 of the "873" series were propagated by cuttings at the Forest Research Institute Nursery in 1971–73.

Single rows of 10 cuttings of each clone were planted in the clonal archive at Rotorua in 1972–74. The clonal rows were maintained as hedges from 1975 to 1980 by regular topping (Libby *et al.* 1972). Judging by the juvenile appearance of the foliage and complete lack of male and female flowers, the hedging was effective in arresting physiological aging of the clones.

The hedges were sprayed annually from 1975 to 1978 to minimise disease, but no spraying was done in 1979. The particularly wet summer of 1979–80 and the lack of spraying contributed to severe infection of the "871" hedges by *D. pini,* first fully evident in April 1980. The "870" and "873" hedges appeared generally less infected than the "871" hedges.

The "871" hedges were assessed on 30 May 1980 and "870" hedges on 22 July 1980 (the "873" hedges were not sufficiently infected to justify assessment). Each hedge was assigned a single score according to a 0 (no infection) to 4 (severe infection) scale. The "870" and "871" clonal series were analysed separately. Clones were grouped into their full-sib families, and individual clone scores analysed in a one-way analysis of variance to estimate components of variance for "families" and "clone-in-families".

Full-sib family means were estimated. It was possible to arrange some of the full-sib families in the "871" series of clones into a factorial mating design, and to estimate general and specific combining ability effects using the method of fitting constants (Searle 1971).

In the "870" series there were 10 full-sib families, with five clones in each. Considerable clone-to-clone variation in resistance occurred **within** families, but families did not differ significantly in mean resistance (Tables 2 and 3). These hedges had only a light average infection (mean score of 1.74), though four clones were assigned maximum scores of 4 (severe infection). Four clones – 4 (ex 89 × 55), 15 (ex 90 × 19), 30 (ex 89 × 97), and 41 (ex 88 × 97) – showed no infection.

TABLE 2—Means and range for infection score (0–4) for the 50 clones of the "870" series grouped into their 10 full-sib families

Rank†	Family	Mean of 5 clones	Clone numbers	Range in 5 clones
1	96 × 55	1.20	16–20	1–2
2	89 × 97	1.20	26–30	0–3
3	97 × 7	1.40	31–35	1–2
4	97 × 55	1.60	36–40	1–3
5	89 × 55	1.60	1–5	0–3
6	121× 19	1.60	46–50	1–3
7	90 × 121	1.80	6–10	1–4
8	90 × 19	2.00	11–15	0–4
9	88 × 97	2.00	41–45	0–4
10	88 × 19	3.00	21–25	2–4
General mean		1.74		

† In order of resistance to Dothistroma needle blight

TABLE 3—Analysis of variance of infection score (0–4) on 10 full-sib families in the 50 clones of the "870" series

Source	d.f.	Mean squares	E(MS)†	F-test
Families	9	1.3800	$\sigma^2_{c:f} + 5\ \sigma^2_f$	1.17 ns
Clones : families	40	1.1800	$\sigma^2_{c:f}$	
Total	49			

† Variance component estimates were: $\hat{\sigma}^2_f = 0.04 \pm 0.13$

$\hat{\sigma}^2_{c:f} = 1.18 \pm 0.26$

The intraclass correlation, defined as

$$h^2_f = \hat{\sigma}^2_f/(\hat{\sigma}^2_f + \hat{\sigma}^2_{c:f}),$$

and measuring the average correlation between clones from the same family in needle blight resistance, was only 0.03.

In the "871" series there were 25 full-sib families, with from three to eight clones in each. Clone-to-clone variation in resistance **within** families was again considerable and of similar magnitude to that recorded in the "870" series but there were also statistically significant differences **between** families (Tables 4 and 5). The intraclass correlation was 0.23.

TABLE 4—Means and ranges for infection score (0–4) for the 101 clones of the "871" series grouped into their 25 full-sib families (three to eight clones per family)

Rank†	Family	Mean	Number of clones	Range
1	99 × 97	0.67	3	0–1
2	101 × 19	0.67	3	0–1
3	121 × 7	1.00	5	0–2
4	108 × 37	1.25	4	1–2
5	97 × 55	1.33	3	0–3
6	99 × 121	1.33	3	1–2
7	88 × 7	1.50	8	0–4
8	96 × 7	1.67	3	0–3
9	97 × 7	1.67	3	1–2
10	121 × 97	1.71	7	1–4
11	89 × 97	1.75	4	1–4
12	88 × 97	1.75	4	1–2
13	80 × 19	2.00	3	1–3
14	55 × 121	2.00	3	1–4
15	89 × 55	2.00	1	2–2
16	88 × 19	2.00	5	1–3
17	90 × 19	2.00	4	1–3
18	109 × 7	2.33	3	1–3
19	88 × 55	2.37	8	0–3
20	112 × 101	2.50	4	1–4
21	89 × 121	2.67	6	2–3
22	96 × 55	3.00	4	2–4
23	90 × 121	3.00	3	2–4
24	80 × 121	3.67	3	3–4
25	88 × 121	3.75	4	3–4
General mean		1.99		

† In order of resistance to Dothistroma needle blight

TABLE 5—Analysis of variance of infection score (0–4) on 25 full-sib families in the 101 clones of the "871" series

Source	d.f.	Mean squares	E(MS)†	F-test
Families	24	2.3758	$\sigma^2_{c:f} + 4.0124\ \sigma^2_f$	2.20**
Clones : families	76	1.0786	$\sigma^2_{c:f}$	
Total	100			

† Variance component estimates were: $\hat{\sigma}^2_f = 0.32 \pm 0.17$

$\hat{\sigma}^2_{c:f} = 1.08 \pm 0.17$

Family 88 × 121 showed extreme susceptibility to infection, the four individual clones having disease scores of 4, 3, 4, and 4. There were no completely immune families though eight individual clones (58, 60, 66, 73, 76, 164, 177, and 198) showed no infection.

From the array of 25 full-sib families, a subset of 14 families was extracted to form an unbalanced 5 × 5 factorial mating design (Table 6). The analysis of variance of the clones in these families (Table 7) provided a test for general combining ability (GCA) and specific combining ability (SCA). The only "850" series parents to show statistically significant GCA effects were both used as males, Clone 7 (resistant) and Clone 121 (susceptible). Specific combining ability effects were negligible.

TABLE 6—Subset of 14 full-sib families of "850" series parents used for combining ability analysis of the "871" series clones

Female parent	Male parent				
	7	19	55	97	121
80	-	X	-	-	X
88	X	X	X	X	X
89	-	-	X	X	X
90	-	X	-	-	X
96	X	-	X	-	-

TABLE 7—Analysis of variance of infection score (0–4) by method of fitting constants of 60 of the "871" series clones grouped into 14 full-sib families to form an unbalanced 5 × 5 factorial design

Source	d.f.	MS	F-test
GCA	8	3.3796	3.27 **
Males (4)		(5.9654)	5.77 **
Females (4)		(0.7939)	0.77 ns
SCA	5	0.3857	0.37 ns
Clones: Full-sib families	46	1.0337	

The ortets of the "870", "871", and "873" clones had been re-examined in 1976, 6, 5, and 3 years respectively after they were first selected. None of the four clones of the "870" series or of the eight clones of the "871" series that showed nil infection by *D. pini* met the minimum acceptable phenotypic standards in volume, straightness, branching, and wood density required for plus-trees. Thus, although the Rotorua clonal hedges were useful for demonstrating variation and inheritance of resistance, no clones were selected for the breeding programme.

Hedges of First-generation Clones at Kaingaroa

Clonal variation in needle blight resistance in hedges of first-generation clones can be more summarily described. A group of 588 *P. radiata* clones known as the "268" series has been maintained in a hedged archive in Cpt 1350 of Kaingaroa Forest since 1971. Most of the clones were established in the archive by grafting.

The ortets themselves were selected in Kaingaroa Forest in stands planted in the period 1953–58. Thus the age of the clones was 13–18 years at time of initial propagation. Allowing for the time lag before hedging commenced, the clones had attained a physiological age of 18–23 years (chronological age of 22–27 years) in 1980 when infection was heavy.

A detailed evaluation of the general combining ability of these clones in progeny tests was made in 1974 and again in 1979, resulting in the selection of 78 clones of proven good performance in growth, stem straightness, branching quality, and with a degree of improvement in wood density and resistance to *Naemacyclus minor* Butin needle cast. The hedges of these 78 clones were assessed for *D. pini* attack in July 1980.

Thirty clones showed a high level of resistance to *D. pini* in relation to infection levels on neighbouring clones:

Nil infection – 109, 201, 229, 424, 452

Slight infection – 58, 61, 62, 65, 91, 131, 142, 149, 194, 228, 248, 323, 345, 357, 363, 377, 386, 395, 405, 426, 459, 494, 547, 589, 609.

The other clones were moderately to severely infected and thus might be bad risks for inclusion in future seed orchards.

DISEASE RESISTANCE IN A DIALLEL PROGENY TEST

The results from the "871" series clones demonstrated that there is quantitative genetic variation in *D. pini* resistance in *P. radiata* with strong indications that general combining ability effects, reflecting additive gene action, are more important than specific combining ability effects. This means that genetically resistant strains could be produced via seed from a multi-clone seed orchard, though the very marked within-family variation (apparently genetic) could be fully exploited only by mass vegetative propagation of resistant clones.

In the N.Z. Forest Service breeding programme for *P. radiata* there are over 1000 selected first- and second-generation plus-trees in progeny tests, either as open-pollinated, polycross, self, or full-sib families. Natural infection of any of these tests by *D. pini* would give the opportunity to examine the genetic variation and inheritance of resistance in material already selected for important traits such as growth, stem form,

and wood properties. Such an opportunity arose in 1980 when a diallel progeny test involving 25 of the "850" series of first-generation plus-trees became infected with *D. pini* after the cessation of spraying the previous year. The parent trees were all selected in the 1950s long before *D. pini* appeared in New Zealand so they can be considered a random sample of *P. radiata* orchard clones with respect to disease resistance.

The 25 parents were randomly grouped into five sets of five, with each set being designated as a "diallel". Parents within each "diallel" were crossed together in a modified half-diallel mating design to form 10 full-sib families per diallel. The five separate diallels, 50 full-sib families altogether, constitute a partial diallel design known as a disconnected diallel.

The progenies were planted out in field tests on 12 sites throughout New Zealand in 1975, but only the results from the test at Cpt 905 in Kaingaroa Forest are reported here. This test is on flat land at an altitude of 530 m and is adjacent to Cpt 901, site of the first heavy outbreak of Dothistroma needle blight in Kaingaroa Forest in 1966. The design comprised six block replicates each of 0.7 ha, with each block replicate divided into five sub-blocks of 0.14 ha. Within each block replicate, each of the five diallels was randomly assigned to one of the sub-blocks. Five trees of each full-sib family per diallel were planted at 5 × 5 m spacing as individually randomised single-tree plots in one sub-block of each block replicate. Trees from an unselected bulk seedlot were planted in each sub-block as a control.

The test was assessed during October 1980 at which time the trees were unpruned, retaining live branches to the ground because of the wide spacing. The site had been sprayed with copper oxychloride in November 1978. Each tree was assigned an infection score on the 0–4 scale. Scores applied only to the bottom half of the tree and related directly to the degree of defoliation and quantity of visibly infected needles. Trees were scored independently and absolutely, i.e., without relationship to infection level on neighbouring trees.

Individual tree scores were analysed in a one-way analysis of variance and a pooled estimate was obtained of the variance "within full-sib families in sub-blocks" (σ^2_w). The harmonic mean (k) of the number of trees per sub-class (i.e., per full-sib family per sub-block) was also calculated.

Arithmetic means were calculated of the sub-classes and a separate analysis of variance was made for each diallel, following the linear model:

$$y_{ijk} = \mu + b_k + g_i + g_j + s_{ij} + e_{ijk}$$

where y_{ijk} is the mean score of the progeny of the i^{th} female and j^{th} male in the k^{th} block replicate; μ is the mean score for the diallel; b_k is the effect of the k^{th} replicate; g_i is the general combining ability (GCA) effect of the i^{th} female; g_j is the GCA effect of the j^{th} male; s_{ij} is the specific combining ability (SCA) of the cross between the i^{th} female and j^{th} male; and e_{ijk} represents an error effect. Griffing's Method 4 (Model I), which assumes GCA and SCA effects are fixed, was followed for the estimation and testing of GCA and SCA effects (Griffing 1956) in each diallel. Parental breeding values, defined as $BV_i = 2g_i + \mu$, were estimated as outlined by Snyder (1975). Cross (i.e., full-sib family) means were estimated directly from the arithmetic mean of the sub-class means.

The 25 parents were ranked for resistance to needle blight infection using what is here defined as "relative breeding value" (RBV), given by

$$\text{RBV} = \frac{\mu}{\text{BV}} \times 100$$

where RBV is the relative breeding value of the i^{th} parent in the n^{th} diallel, BV is its breeding value determined within its diallel, and μ is the diallel mean. RBV adjusts for possible environmental influences on the differences between diallels in mean infection score which could bias direct contrasts between unadjusted breeding values of parents in different diallels. This method of adjustment assumes that the average breeding value of the parents is the same in each diallel.

In a similar fashion, the 50 full-sib families were ranked for disease resistance using "relative cross value" (RCV), given by

$$\text{RCV} = \frac{\mu}{\text{CM}} \times 100$$

where RCV is the relative cross value of the ij^{th} family in the n^{th} diallel, CM is the unadjusted full-sib family or cross mean, and μ is the diallel mean. As with RBV, RCV adjusts for environmental differences between diallels.

Additionally, disease infection scores on the 0–4 scale were transformed into binomial data by assigning trees with scores of 0–2 a value of 0, and trees with scores of 3–4 a value of 100%. Family means of the transformed scores were calculated in an attempt to express disease resistance as the "percentage of trees badly infected by *D. pini*".

The 30 sub-class means of the unselected control trees were analysed in a two-way analysis of variance to measure the environmental effects associated with block replicates and diallels.

For the purpose of estimating GCA and SCA variance components, Griffing's Method 4 (Model II) was followed (Griffing 1956). This entails no change to the mean squares in the analysis of variance in each diallel, though their expected values differ to allow for the assumption that GCA and SCA effects are now random. Sums of squares for GCA and SCA from the individual diallels were pooled to give an over-all analysis, and estimates of GCA and SCA variance components based on all 50 full-sib families and all 25 parents.

General and Specific Combining Ability Effects

A very consistent pattern of highly significant GCA effects, and only weak or non-significant SCA effects showed up in each of the diallels (Table 8). Thus in each diallel there were some parent clones of the "850" series that were either more susceptible or less susceptible than the diallel average. An additive mode of gene action for disease resistance is therefore evident.

A ranking of all 25 parents based on relative breeding values for infection score is given in Table 9. Also shown are the unadjusted breeding values for both disease score and percentage of trees badly infected, and the GCA effects for disease score.

TABLE 8—Analysis of variance of sub-class means, and F-tests of infection score (0–4) for general and specific combining ability effects in five disconnected modified half diallels

Source	d.f.	Mean squares					
		Diallel 1	Diallel 2	Diallel 3	Diallel 4	Diallel 5	All diallels
Reps	5	0.3935	0.8075	0.6459	0.9280	1.2571	
GCA	4	0.7613	1.8768	0.6876	1.2943	0.9051	1.1050
SCA	5	0.3084	0.2100	0.2284	0.1611	0.0238	0.1864
Error	45	0.0996	0.1400	0.1651	0.1301	0.1474	0.1364
F-test, GCA		7.64**	13.41**	4.16**	9.95**	6.14**	8.10**
F-test, SCA		3.10*	1.50 ns	1.38 ns	1.24 ns	0.16 ns	1.37 ns
Mean score, families		1.99	2.13	1.49	1.68	2.14	1.90
Mean score, control†		2.03	1.44	1.45	1.67	2.01	1.72
Mean percentage badly infected trees, families		25	33	11	14	32	23
Mean percentage badly infected trees, control†		21	9	3	10	32	15

† Unselected seedlot

The most significant GCA effects were detected in Diallels 2 and 4, with progeny of Clones 37 and 87 exhibiting very high disease resistance and those of Clones 88 and 117 showing very poor disease resistance.

The almost complete absence of significant SCA effects meant that the performance of single crosses (full-sib families) was predictable from the GCA effects of the two parents. There were some exceptions in Diallel 1 where some crosses, notably 97 × 7 and 97 × 121, were respectively more or less susceptible than predicted. A ranking of all 50 full-sib families based on relative cross value for infection score is given in Table 10. The mean percentage of badly infected trees ranged in one of the diallels from 3% in Cross 37 × 99 to a massive 69% in Cross 88 × 96.

Comparison of Families with Control

The means of the 10 families and of the bulk control seedlot are shown for each diallel at the foot of Table 8. There were significant **environmental** differences between diallels ($F_{4,20=\ 6.5\ **}$) reflected by difference in the mean score (L.S.D. = 0.35 between diallels) of the control. Diallels 1 and 5 were more heavily infected than Diallels 2–4.

With the exception of Diallel 2, the mean infection levels of the control and crosses were similar. Diallel 2 was anomalous in that the control lot was only lightly infected (mean score 1.44) while the crosses were quite heavily infected (mean score 2.13). Such a difference could well have occurred by chance though there is a strong possibility that the high average disease score of the crosses was inflated by one exceptionally susceptible parent (Clone 88).

TABLE 9—General combining ability rankings for disease resistance of 25 parents of the "850" series (based on relative breeding value)

Rank†	Parent	Diallel	Relative breeding value (%)	Within diallel GCA		
				Breeding‡ value	GCA effect§	Breeding value as percentage of trees badly infected‖
1	37	2	184	1.1556	-0.4856*	-12
2	87	4	173	0.9666	-0.3567*	0
3	100	5	153	1.3945	-0.3711*	7
4	98	3	138	1.0778	-0.2044*	2
5	7	1	134	1.4889	-0.2522*	13
6	91	3	132	1.0778	-0.2044*	6
7	119	4	122	1.3778	-0.1511	1
8	19	1	120	1.6557	-0.1688*	17
9	99	2	114	1.8667	-0.1300	22
10	81	5	105	2.0389	-0.0489	32
11	93	4	101	1.6556	-0.0122	4
12	89	3	100	1.4889	0.0011	7
13	55	1	96	2.0779	0.0423	23
14	120	5	91	2.3500	0.1067	47
15	80	2	90	2.3667	0.1200	35
16	96	2	89	2.3889	0.1311	55
17	191	5	88	2.4167	0.1400	36
18	121	1	87	2.2889	0.1478*	25
19	110	5	86	2.4834	0.1733*	38
20	82	3	83	1.7889	0.1511	12
21	97	1	81	2.4557	0.2312*	45
22	108	4	79	2.1333	0.2267*	26
23	90	3	76	1.9556	0.2344*	28
24	117	4	74	2.2667	0.2933*	40
25	88	2	74	2.8556	0.3644*	66

† From most resistant to most susceptible.

‡ Based on 0–4 scale disease score.

§ Effects marked * are statistically significant, with estimates exceeding $\pm$ 2 S.E.

‖ Least squares method of estimation yielded negative value for the highly resistant parent, 37.

TABLE 10—Full-sib family rankings for disease resistance at age 5 years of 50 crosses among "850" series parents (based on relative cross value)

Rank†	Cross F	Cross M	Diallel	Relative cross value (%)	Within diallel Cross‡ mean	Within diallel SCA§ effect	Percentage of trees in family badly infected
1	91	98	3	156	0.9500	-0.1501	3
2	37	99	2	156	1.3667	-0.1444	3
3	19	7	1	144	1.3833	-0.1890*	7
4	98	89	3	133	1.1167	-0.1667	3
5	87	93	4	133	1.2667	-0.0444	3
6	37	96	2	133	1.6000	-0.1722	17
7	100	81	5	129	1.6500	-0.0667	26
8	119	87	4	126	1.3333	0.1611	10
9	55	7	1	122	1.6333	-0.1501	10
10	80	37	2	113	1.8833	0.1222	19
11	100	120	5	112	1.9000	0.0277	29
12	117	87	4	111	1.5167	-0.0999	15
13	110	100	5	111	1.9167	-0.0222	15
14	87	108	4	110	1.5333	-0.0167	7
15	100	191	5	109	1.9667	0.0611	21
16	119	108	4	106	1.5833	-0.1723	3
17	121	7	1	105	1.9000	0.0111	21
18	119	93	4	104	1.6167	0.1000	10
19	91	90	3	103	1.4500	-0.8889	12
20	91	82	3	99	1.5000	0.0444	16
21	91	89	3	99	1.5000	0.1944	4
22	121	19	1	99	2.0167	0.0444	23
23	89	82	3	98	1.5167	-0.1222	7
24	55	19	1	98	2.0333	0.1665	30
25	97	19	1	98	2.0333	-0.0224	27
26	119	117	4	97	1.7333	-0.0889	14
27	88	37	2	97	2.2000	0.1945	24
28	80	99	2	97	2.2000	0.0833	31
29	191	81	5	96	2.2333	0.0055	33
30	81	120	5	95	2.2500	0.0555	37
31	88	99	2	94	2.2667	-0.0944	42
32	110	81	5	94	2.2667	0.0056	32
33	97	55	1	93	2.1333	-0.1335	30
34	97	121	1	93	2.1333	-0.2390*	30
35	99	96	2	93	2.2833	0.1555	39
36	191	120	5	93	2.3000	-0.0834	38
37	98	90	3	91	1.6333	0.1166	17
38	98	82	3	91	1.6333	-0.1999	7
39	80	96	2	91	2.3333	-0.0445	39
40	108	93	4	90	1.8667	-0.0278	13
41	110	120	5	88	2.4167	0.0000	48
42	117	93	4	87	1.9333	-0.0278	15
43	110	191	5	87	2.4667	0.0167	42
44	121	55	1	87	2.3000	0.1166	27
45	88	80	2	87	2.4500	-0.1611	46
46	97	7	1	87	2.3000	0.3277*	43
47	90	82	3	85	1.7500	-0.1222	16
48	89	90	3	82	1.8167	0.0945	24
49	88	96	2	79	2.6833	0.0611	69
50	108	117	4	70	2.4167	0.2167*	51

† From most resistant to most susceptible.
‡ Based on 0–4 scale disease score.
§ Effects marked * are statistically significant, with estimates exceeding ± 2 S.E.

Variance Components

Analysis of variance of the five diallels combined is shown in Table 11. Of the combining ability variances, only σ^2_{gca} was statistically significant, the estimate of the component being nearly 6 times greater than that of σ^2_{sca}. The components of variance estimated in the experiment are shown in Table 12. It can be seen that 6.4% of all the tree-to-tree variation in infection score in the test can be attributed to GCA effects. There was a very large within-subclass component (σ^2_w) representing genetic variance within full-sib families plus environmental and chance infection variance within sub-blocks.

Implications for Heritability of Resistance

An estimate of 0.30 was obtained for narrow-sense heritability of Dothistroma needle blight resistance from:

$$h^2 = \frac{4\,\hat{\sigma}^2_{gca}}{\hat{\sigma}^2_{gca} + \hat{\sigma}^2_{sca} + \hat{\sigma}^2_{p} + \hat{\sigma}^2_{w}}$$

The denominator (phenotypic variance among individual trees) does not include sub-block error, replicate, or diallel components of variance since it is probable that in an actual selection operation to find resistant trees these sources of variance could be controlled by using neighbour tree comparisons (Ledig 1974). Since general combining ability was by far the most important kind of genetic variance

$$(\hat{\sigma}^2_{gca}/\hat{\sigma}^2_{gca} + \hat{\sigma}^2_{sca} = 0.85),$$

the effective heritability could probably be increased by reducing the size of the within-subclass variance (σ^2_w). This could be achieved mainly by eliminating environmental differences between neighbouring trees or between trees in the same sub-block, as would tend to occur as general infection levels in the stand increased. A closer spacing between trees than the 5 × 5 m in this test would also reduce chance and environmental differences in infection between trees. The mean disease score of 1.90 (23% of trees badly infected) represented only a modest infection level in this test but was still sufficient to reveal genetic differences between families and provide a satisfactory screening.

Relationship Between Parent and Progeny Susceptibility

Fourteen of the clones of the "850" series studied in the diallel test had been planted in 1960–63 as grafts in the Rapanui seed orchard of N.Z. Forest Products Ltd. This presented the opportunity to measure the parent-progeny correlation for *D. pini* susceptibility, although there are doubts about the reliability of clonal performance as a predictor of progeny performance. Be that as it may, the existence of a high correlation between grafted clones and their seedling offspring for a trait such as disease resistance would provide considerable scope for rapid selection and development of resistant varieties.

The grafts were assessed for susceptibility in early September 1966. An average of 139 ramets per clone was assessed for defoliation on a 0–7 scale as follows: 0 = no

TABLE 11—Analysis of variance of subclass mean infection scores (0-4) in disconnected diallel (random effects model)

Source	df	SS	MS	E(MS)
Reps	5	7.2891	1.4578	$\sigma^2_w/4.46 + \sigma^2_p + 10\ \sigma^2_{rd} + 50\ \sigma^2_r$
Diallels	4	20.0502	5.0126	$\sigma^2_w/4.46 + \sigma^2_p + 10\ \sigma^2_{rd} + 6\ \sigma^2_{sca} + 48\ \sigma^2_{gca} + 60\ \sigma^2_d$
Reps × diallels	20	12.8702	0.6435	$\sigma^2_w/4.46 + \sigma^2_p + 10\ \sigma^2_{rd}$
Crosses in diallels				
GCA	20	22.1004	1.1050	$\sigma^2_w/4.46 + \sigma^2_p + 6\ \sigma^2_{sca} + 18\ \sigma^2_{gca}$
SCA	25	4.6589	0.1864	$\sigma^2_w/4.46 + \sigma^2_p + 6\ \sigma^2_{sca}$
Reps × crosses : diallels (= error)	225	30.7006	0.1364	$\sigma^2_w/4.46 + \sigma^2_p$
Total	299			
Within rep-cross subclasses (from raw data)	1084	635.6667	0.5864	σ^2_w

TABLE 12—Variance components for infection score (0–4) estimated in the disconnected diallel test

Component	Description	Estimate ± S.E.	Percentage contribution to total variance
σ^2_{gca}	GCA variance (additive genetic effects)	0.0480 ± 0.0186	6.4%
σ^2_{sca}	SCA variance (non-additive genetic effects)	0.0083 ± 0.0087	1.1%
σ^2_r	Variance among block replicates (environmental variance caused by patchiness of infection and/or zonation of the test site	0.0163 ± 0.0161	2.2%
σ^2_d	Variance among diallels (environmental differences confounded with genetic differences between diallels)	0.0336 ± 0.0484	4.5%
σ^2_{rd}	Variance among sub-blocks ("large" plot error, or sub-block environmental variation within block replicates caused by patchiness of infection)	0.0507 ± 0.0194	6.8%
σ^2_p	Variance due to interaction between full-sib families and block replicates	0.0049 ± 0.0140	0.6%
σ^2_w	Within sub-class variance (genetic variance within full-sib families + environmental or chance differences in infection from tree-to-tree within subclasses)	0.5864 ± 0.0252	78.4%
σ^2_T	Total variance	0.7482	

infection; 1 = trace to 5%;2 = 6 to 25%; 3 = 26 to 50%; 4 = 51 to 75%; 5 = 76 to 95%; 6 = 96 to 100%; 7 = dead due to defoliation. The average score for the orchard was 0.58, indicating only light infection. The mean score and mean percentage of non-infected grafts (i.e., with scores of 0) were calculated for each clone.

Parent-offspring correlations were calculated between mean score and percentage of non-infected grafts in the seed orchard in 1966 and relative breeding value of the clones as measured in the diallel progeny test at Kaingaroa in 1980 (Table 9). The correlations were highly significant: –0.68 for clonal score / progeny and 0.67 for clonal percentage of non-infected trees / progeny. A plot of clonal percentage of non-infected grafts *versus* relative breeding value measured on progeny is given in Fig. 1.

Even with the light infection levels occurring in the Rapanui seed orchard, the correlations were sufficiently strong for clonal performance in the seed orchard to have been used as a fairly reliable measure of general combining ability. Only in clone 37

was progeny performance far out of line (*see* Fig. 1) with clone performance. Heritability, based on the correlation coefficient of 0.67 and using the adjustment procedure described by Franklin (1975), was estimated to be approximately 0.50.

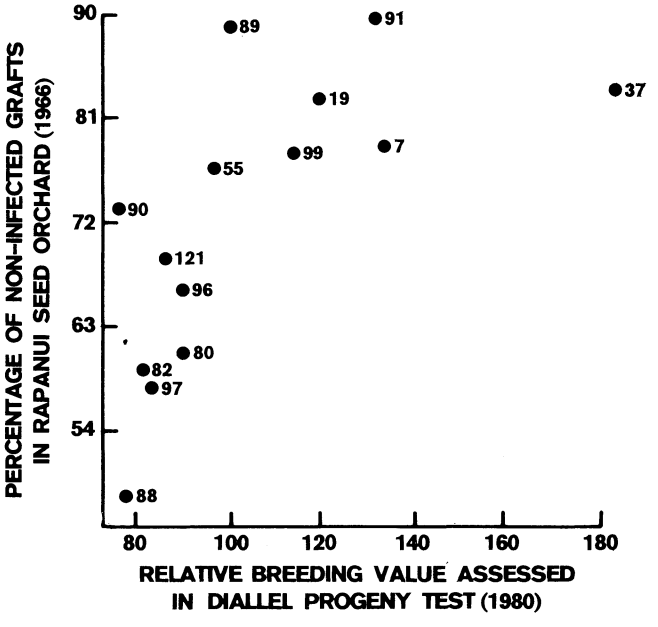

FIG. 1—Plot of clonal values for percentage of grafts infected by **D. pini** at Rapanui seed orchard (1966) **versus** general combining ability as measured by relative breeding value on seedling progeny (1980).

GENERAL DISCUSSION AND CONCLUSIONS

These results give considerable hope for the breeding of strains of *P. radiata* with improved resistance to Dothistroma needle blight. Phenotypic variation in resistance is high and easy to assess; genetic variation is essentially additive, with a potentially high heritability; screening to identify resistant individuals or families can be effectively done in well-designed field tests, favourably located and managed to encourage natural inoculation; and seed can be mass-produced in clonal seed orchards.

In these studies, resistance to infection behaved as a quantitative (polygenic) trait. This is what plant pathologists (e.g., Gibson *et al.* 1980) have sometimes called "horizontal" resistance, assumed to be controlled by large numbers of genes each with small effects rather than by just one or a few major genes as in so-called "vertical" resistance. Resistant strains of trees should therefore be robust against racial variation and genetic mutations in the pathogenicity of the fungus.

The strong additive genetic variance (general combining ability) and moderate heritability of disease resistance shown particularly in the diallel test indicate that the straightforward procedure of selecting resistant trees and allowing them to inter-mate randomly in a seed orchard would give predictable improved resistance in the offspring.

Furthermore, recurrent selection for resistance should effectively accumulate and concentrate alleles and lead to progressively greater resistance over successive generations of breeding. The main constraints to achieving economically significant resistance are the usual limitations of restricted selection differentials and perverse genetic correlations that may handicap any breeding programme with multiple-trait selection objectives. Clone 37, for example (*see* Table 9), showed a very high breeding value for disease resistance, but in numerous other tests its progeny have consistently shown such poor growth and such a high incidence of stem malformation that all grafts of it have been removed from seed orchards. Its valuable genes for resistance to needle blight cannot therefore be utilised in the current breeding programme. Nevertheless, there are other seed orchard clones of the "850" series (the present source of seed orchard seed), notably 7, 19, 87, 91, 98, and 100 (*see* Table 9), whose progeny showed above-average resistance and that could be used as the basis of a resistant variety. The simple operation of collecting seed from these six clones in the seed orchards and segregating it for use in areas of high disease hazard is a practical measure that could be implemented immediately. In the longer term, special seed orchards in which extra emphasis is given to disease resistance (but probably at the expense of diminished gains in other traits) would be even more effective. To this end, a provisional list of suitable clones has been compiled.

There are currently 78 *P. radiata* clones of the "268" series (Kaingaroa forest selections) re-selected from 588 candidates on the basis of 10-year progeny test results that are providing the bulk of the stock for new seed orchards. Some of these show promising disease resistance. In addition, 84 second-generation clones of the "875" series are being increasingly used in seed orchards. There is a good likelihood of finding a disease-resistant group of clones among these, using existing progeny tests, the seed orchards, and clonal archives for screening. The alternative approach of selecting for resistance in genetically unimproved stands, as was done in the "867" series of clones, appears inefficient as two generations of intensive selection for other commercially important traits are lost. Nevertheless, the "867" series programme did yield five resistant clones with sufficiently promising growth and form to be used in seed orchards.

Scion material of 21 clones of "field-resistant" trees selected in Kenya and Tanzania (Ivory & Paterson 1970) was sent to New Zealand in 1968 to supplement the breeding programme (Forest Research Institute 1968). These clones have been maintained in clonal archives as "hedges", but in New Zealand have neither been evaluated for disease resistance nor tested for suitability as parents in seed orchards. The breeding programme in Kenya, however, has progressed as far as progeny testing of "resistant" plus-trees and establishment of clonal seed orchards with the best clones (W. G. Dyson, Tree Breeder 1960–77, E.A.A.F.R.O., Kenya, pers. comm.). Results from clonal testing in Kenya (Ivory & Paterson 1970) indicated that seven of the clones sent to New Zealand were especially disease-resistant. The parents were selected for good growth, branching, and stem straightness, as well as for disease resistance. These clones could consequently be used directly in specialty "disease-resistant" orchards though their value in New Zealand is doubtful until tested.

Mass propagation of selected resistant clones by cuttings, and of selected resistant full-sib or half-sib families by seed/tissue-culture/fascicles/cuttings are options that could be pursued if suitable methods can be developed (*see* Smith *et al.* 1981). The

strong general combining ability effects for resistance, however, indicate that such elaborate methods are unlikely to give much more improvement than a seed orchard of the best parents unless within-family variation can be exploited by clonal selection.

Progeny tests on uniform sites with uniformly heavy infection should provide good opportunities for future selection. Clonal tests (including clonal seed orchards and clonal archives) are apparently reliable for assessing the disease resistance of individual trees and of their general combining ability (i.e., the likely resistance of their seedling progeny). Results from the young "871" series clones studied in hedges showed that clonal variation in resistance did reflect combining ability differences among the parents. Resistant clones in turn can therefore be expected to yield a high frequency of resistant seedling offspring.

Clonal variation in disease resistance was particularly well expressed and easy to assess in the clonal hedges, though it was unfortunate that the most resistant clones were from ortets of low standard for growth and form. The periodic removal of the uninfected shoots in the hedging process accentuates the clonal differences. In addition, it is possible that juvenile hedges actually exhibit greater extremes of susceptibility and resistance to infection than do normal trees (cf. Heather *et al.* 1980).

Mechanisms underlying the observed genetic variation in resistance of *P. radiata* to Dothistroma needle blight are presently under study at the Forest Research Institute. There is some evidence that variation in resistance relates to substances on the needle surface that inhibit germination of spores and/or prevent penetration of hyphae through the stomatal pores (Forest Research Institute 1977a). A more promising explanation resulting from research by Dr L. Shain (of the University of Kentucky, United States) and Dr R. Franich at the Forest Research Institute, Rotorua, is that genetic variation in resistance in *P. radiata* could be a reflection of variation in the sensitivity to dothistromin, the cause of the brick-red necrotic lesions in the needles (Forest Research Institute 1980; Shain & Franich 1981).

In the tests described in this paper, the pathogen concentrated on was *D. pini*. *Pinus radiata* also suffers seasonal defoliation in New Zealand from infection by another needle-cast fungus, *Naemacyclus minor* (Forest Research Institute 1977b; Gibson 1979) which in turn may be associated with *Lophodermium pinastri* (Schrad.) Chev. (Gilmour 1964). Genetic variation in resistance to *N. minor* needle cast has been recorded many times in progeny tests (e.g., Wilcox *et al.* 1975) and, like *D. pini* resistance, shows a strongly additive mode of inheritance, with a comparatively high heritability. Clonal variation in resistance is also strong (Forest Research Institute 1977b). The relative ease with which progeny and clonal tests become infected with *N. minor,* causing marked family and clonal differences in needle cast and chlorosis, raises the possibility of using *N. minor* needle cast as an index of *D. pini* susceptibility. It has been generally observed in diseased progeny and clonal tests of *P. radiata* in the Rotorua area that the pathogen responsible is predominantly either *D. pini* or *N. minor* but not both, and it is possible that joint attack is prevented by interactions between the two fungi. It has been demonstrated, for example, that *D. pini* inhibits the growth of *N. minor* in culture (Osorio & Rack 1980). There is nevertheless some suggestion of a positive genetic correlation between susceptibility to the two diseases. Clone 850-121 was the most susceptible parent to *N. minor* needle cast in a factorial test (Wilcox *et al.* 1975) and

also showed high susceptibility to *D. pini* in the diallel test (as 5-year-old seedling trees) and in the "871" series clonal test (as hedged cuttings). Furthermore, Clone 850-121 is highly susceptible as a graft to *N. minor,* and its self-pollinated offspring also show extreme susceptibility. Further studies are planned to determine the reliability of *N. minor* needle cast defoliation scores in progeny tests for indirect selection for Dothistroma needle blight resistance.

There are insufficient data available to determine what the level of *D. pini* resistance obtainable by breeding will mean in economic terms. Exposure to infection of a wide range of *P. radiata* families of various ages is needed to establish the full range in tolerance of the species. Considering the size of the spraying programme, sufficient resistance to eliminate the need to spray, to increase the effectiveness of spraying, or to reduce the frequency of spraying are all worthwhile goals.

SOME APPLICATIONS

Any attempts to breed for Dothistroma needle blight resistance will be at the expense of genetic improvement in most other traits. Nevertheless, positive steps that can be taken to incorporate resistance into the breeding programme for *P. radiata* are:

- Collection of seed immediately from the most resistant clones of the "850" series in seed orchards at Gwavas, Waimihia, Kaingaroa, and Te Teko, and segregation of this seed for use on the most vulnerable sites.

- Establishment of special disease-resistant seed orchards, based on selection for general combining ability, starting with the 48 clones which have been provisionally selected. Seed would not be available in commercial quantities until the 1990s.

- Selection of further resistant clones ("268" and "875" series) using existing progeny tests, clonal archives, and the clonal orchards themselves, for use in new orchards.

- For the longer term, controlled breeding should be carried out among the best resistant clones for future testing and selection, and the potential of the recently introduced native *P. radiata* populations should be fully explored.

ACKNOWLEDGMENTS

Thanks are due to B. Bay of N.Z. Forest Products Ltd for providing the assessment data from the Rapanui seed orchard; to A. Firth for the assessment data of the "268" series clones at Kaingaroa; and to Dr C. J. A. Shelbourne, I. J. Thulin, D. R. Briscoe and J. D. Tombleson, and several other members, past and present, of the Genetics and Tree Improvement group in Rotorua, for contributing to various aspects of this work. I. J. Thulin organised the importation of the Kenyan clones.

Drs C. J. A. Shelbourne and S. Chou made helpful comments on the manuscript.

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