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Detection and verification of quantitative trait loci for resistance to Dothistroma needle blight in *Pinus radiata*

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Abstract Six related radiata pine (*Pinus radiata*) full-sib families were used to detect and independently verify quantitative trait loci (QTLs) for resistance to Dothistroma needle blight, caused by *Dothistroma septospora*. The detection families had from 26 to 30 individuals each, and had either a common maternal (31053) or paternal (31032) parent; one family (cross 4) consisted of progeny from both parents, 31053×31032. Approximately 200 additional progeny from cross 4 were clonally replicated and planted at two sites, with at least five to seven ramets of each individual per site. Marker segregation data were collected from a total of 250 RFLP and microsatellite markers, and single factor ANOVAs were conducted separately for each family and marker. A number of putative associations were observed, some across more than one family. Permutation tests were used to confirm expected probabilities of multiple associations based on chance alone. Seven markers representing at least four QTLs for resistance to *Dothistroma* were identified as being significant in more than one family; one of these was significant at $P<0.05$ in three families and highly significant at $P<0.01$ in a fourth. Further confirmation was obtained by testing those markers that were significant in more than one of the detection families (or highly significant in cross 4) in the clonally replicated progeny from cross 4. Four QTL positions were verified in the clonal populations, with a total percent variation accounted for of 12.5.

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Introduction

Dothistroma needle blight, caused by *Dothistroma septospora* (Dorog.) Morelet, is the most serious disease affecting radiata pine (*Pinus radiata* D. Don) in Australia and New Zealand. The disease is characterized by needle necrosis, defoliation of as much as 80% of the tree crown, and a consequent loss of growth. About one fourth of Australia's radiata pine plantations are likely to be in regions where climatic conditions are highly conducive to Dothistroma needle blight with more than half of this in New South Wales (NSW).

Radiata pine is generally thought to be susceptible to Dothistroma infection between the age of 1 and 15 years (Carson 1989). Research in New Zealand (van der Pas 1981) demonstrated that wood volume loss was proportional to needle blight disease intensity for young stands; for example, approximately 50% increment loss resulted from an average disease level of 50%. Growth losses in unsprayed stands, subject to the disease in New Zealand, have been estimated at 6.0 m^2 basal area ha^{-1} over 5 years (Woollons and Hayward 1984). Similar results have been obtained in Australia where it was estimated that for every 1% loss of foliage above 25%, there was a 1% loss in volume growth for up to 75% foliage loss (Old and Dudzinski 1999).

The disease can be managed by aerial spraying with copper oxychloride; however, this can be costly and may have environmental implications. Breeding for quantitative or polygenic resistance is an alternative method of control. Breeding programs in New Zealand have been conservatively estimated to have reduced crown defoliation caused by *Dothistroma* from 20% to 7% (Carson 1989). The aim is to reduce expression of disease symptoms below the threshold level requiring spraying in high-risk areas. Selection for resistance to Dothistroma needle blight has also been a priority in Australia since the early 1980s. A reliable glasshouse test for assessing resistance to *Dothistroma* has not been developed. Field trials are therefore necessary, and it is often difficult to

obtain uniform infection and environmental conditions conducive for the development of disease symptoms.

Development of genetic markers and linkage maps have provided tools for tagging quantitative trait loci or QTLs (Sewell and Neale 2000). Identification of marker loci associated with disease resistance can facilitate dissection of the genetic components of resistance. Indirect selection based on marker genotype is also a possibility. Immediate gains in resistance can be achieved by clonal propagation of seedlings selected within families. In forest trees, QTLs affecting various traits have been reported in pines, eucalypts, and poplars (Groover et al. 1994; Bradshaw and Stettler 1995; Grattapaglia et al. 1995; Byrne et al. 1997a, 1997b; Emebiri et al. 1997; Sewell et al. 2000; Jermstad et al. 2001). However, few of these relationships have been confirmed or verified in an independent population.

The number of progeny per family required for QTL detection and verification in forest trees is generally thought to be quite large (Carson et al. 2003a, 2003b; Devey et al. 2003). In some cases, it may be possible to use several related families of much smaller numbers to accomplish this result (Muranty 1996). Clonal replication is also a possibility in some species and may be used to increase the power of detection (Bradshaw and Forster 1992).

The specific objective of this study was to identify markers associated with Dothistroma needle blight resistance loci in *P. radiata*. Two forms of verification were investigated, one using related full-sib families and the other using clonally propagated material from one of the families used for QTL detection.

Materials and methods

Plant materials

The QTL detection families were part of a larger *Dothistroma* screening trial, "Radiata Progeny Trial A" planted near Myrtleford, Victoria (VIC) in 1991. The trial consisted of individual tree plots in 30 replications. The seven families used in this study were from a factorial cross of 4 females × 4 males; the pedigree structure, including grandparents where known, is presented in Fig. 1. Crosses 1 to 4 had 31053 as a common maternal parent, and crosses 4 to 7 had 31032 as a common paternal parent. Cross 4 consisted of progeny from both parents, 31053 × 31032. Each cross had up to 30 individuals, except for cross 5, which was eventually discarded due to more than 70% of the progeny being of incorrect parentage, as indicated from marker genotyping. There were also two other factorial crosses included in the trial, plus controls and extras, giving a total of 80 entries/replication. An estimate of narrow sense heritability was calculated for each factorial as 4×GCA/2×GCA+SCA+error.

As further confirmation of the results obtained for QTL detection, additional seed from cross 4 was obtained, and a large number of progeny were clonally replicated and planted at two locations. The first location was planted in 1994 near Myrtleford, VIC, and the second location was planted in 1995 near Bondo, NSW. Approximately 200 progeny were replicated from five to seven times at each site.

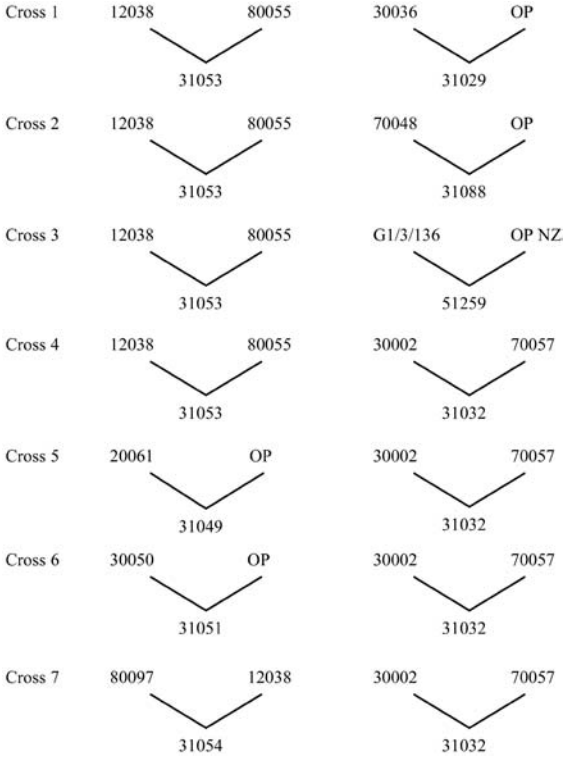

Fig. 1 Parents and grandparents (where known) of seven full-sib families of *Pinus radiata* used to identify QTLs for resistance to Dothistroma needle blight. Crosses 1 to 4 have a common maternal parent (31053) and crosses 4 to 7 have a common paternal parent (31032). Cross 5 was not used due to a high proportion of rogue progeny

Disease assessment

Radiata Progeny Trial "'A" was evaluated for resistance to *Dothistroma* in 1994 by Victorian Plantations Corporation (now called Hancock Victorian Plantations) and the Southern Tree Breeding Association (STBA); the complete set of data was provided by STBA. For assessment of the level of infection, every effort was made to separate effects of diseases or agents other than *Dothistroma*, which could cause similar symptoms. An 11-point visual scoring system was used where 0 = <5%, 1 = 5–15%, to 9 = 85–95%, 10 = >95% of the crown showing symptoms of infection with *Dothistroma*.

The clonal populations at Bondo and Myrtleford were evaluated by the authors. The trees were assessed in December 2000 and January 2001 using a scale of 1–6, with 1 being little or no symptoms of disease and 6 being severe defoliation to about 4–6 m, and affected whorls with 80% to 100% needle blight.

Markers and genetic linkage mapping

Needle samples for DNA extractions and marker assays were collected from grandparents, parents, and progeny. DNA isolation and RFLP procedures were as described previously (Devey et al. 1996). PCR conditions for microsatellite primers were as follows: DNA (50 ng) was amplified in a total volume of 10 µl containing 0.2 µM of each primer, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.8 to 3.0 mM $MgCl_2$, 100 µM of each dNTP, 1 mg/ml BSA, and 0.4 U *Taq* DNA polymerase (Invitrogen). Samples were processed through 30 cycles of 10 s at 94°C, 30 s at 55°C, 30 s at 72°C and a final extension of 72°C for 10 min. One of three annealing temperatures (50, 55 or 60°C) was used depending on pre-

Table 1 Probabilities for the random occurrence of one or more putative associations in six families [Pr(x)], assuming marker and QTL are independent

Probability level	Number of families	Pr(1)	Pr(2)	Pr(3)	Pr(4)	Pr(5)	Pr(6)
0.05	6	0.232134	0.030544	0.002143	0.000085	0.000002	0.000000
0.01	6	0.057059	0.001441	0.000019	0.000000	0.000000	0.000000

determined conditions for each primer pair (Devey et al. 2002). Primers were designed with different fluorescent dye labels for in situ labeling of PCR products. Aliquots of the PCR reaction were combined with deionized formamide and an internal-lane size standard for analysis on an ABI PRISM 310 Genetic Analyzer.

A genetic linkage map had been previously constructed using 96 progeny from the 31053×31032 cross based on segregation of RFLP and microsatellite markers (Devey et al. 1996,1999).

QTL analysis

In the QTL detection families, phenotypic disease scores (D) were adjusted for replication and row within replication, with mean = 0. Adjusted progeny data for each family were grouped by marker genotype based on the ANOVA model:

$$D_{ij} = \mu + G_i + \varepsilon_{ij} \quad (1)$$

where, D_{ij} = individual adjusted disease score, μ = overall mean, (zero), G_i= ith genotypic class (i = 1 to 4), ε_{ij}= error associated with the jth individual in the ith genotypic class.

Analyses were performed separately for each marker in each family. QTLs were verified among families where significant (P<0.05) or highly significant (P<0.01) differences in genotypic class means were observed in more than one family. The probabilities for this occurring based on chance alone can be calculated from the binomial formula:

$$Pr_{(x \text{ out of } n)} = \frac{n!}{x!(n-x)!} * p^x * (1-p)^{n-x} \quad (2)$$

where, n = the number of families, x = the number of putative associations, and P = the probability level, 0.05 or 0.01 (Table 1). To confirm these findings, a simulation was carried out using pseudorandom numbers generated from the GRANDOM procedure in the statistical package GENSTAT.

In the clonal populations, phenotypic data were adjusted for row, column, and replication. Three separate analyses were carried out to detect marker/QTL associations for each marker locus: among alleles segregating in the male parent, among alleles segregating in the female parent and among all possible marker genotypes (maximum of four). Percentage variation accounted for was calculated as: 100×(1−residual m.s.)/(total m.s.).

Results

Marker/trait data

Seven parents were involved in six full-sib crosses to evaluate resistance to Dothistroma needle blight (Fig. 1). A total of 202 progeny were genotyped with 250 markers, including 226 RFLP and 24 microsatellites. Restriction fragments for loci and alleles were carefully matched up among the different families for linkage and QTL analysis; for example, allele 1 in cross 1 would be numbered the same in all six crosses. For multiple loci revealed by the same RFLP probe, the different loci were designated with a different letter, for example *1021a* and *1021b*.

Dothistroma infection/disease data for the QTL detection families showed a normal distribution for each cross. A narrow-sense heritability estimate of 0.18 for resistance was calculated for the factorial cross which included the six full-sib detection families.

In outbreeding species, marker-QTL phase relationships are not known a priori because of linkage equilibrium; also, interactions among QTL alleles are possible depending on the genetic background. Therefore, it was necessary to analyze each marker and family separately. For six families and 250 markers, this resulted in 1,500 ANOVAs. Forty-seven marker/family analyses showed significance at the 5% level, and 17 showed significance at the 1% level. These numbers are not substantially different than what would be expected based on chance alone, e.g., 15 false positives (type I errors) are expected at P<0.01 with this number of analyses. It was therefore likely that most of the associations were false positives.

The probabilities of one or more putative associations in six families, assuming marker and QTL are independent are listed in Table 1. For example, the probability of observing two associations at the 5% level of error probability is expected to be P= 0.031. To confirm these expectations, an empirical test was carried out using permutated phenotypic data generated with the same mean (−0.13889) and variance (0.416678) as the actual adjusted *Dothistroma* data. Using the observed genotypic data from 250 markers, 1,500 analyses of variance were carried out with 1,000 randomly generated phenotypic data sets. The results are presented in Table 2. It can be seen that the number of significant analyses were smaller than the expectations calculated from the probabilities given in Table 1. This may be because some of the marker loci are linked or because some of the marker loci were uninformative in that the marker was not segregating. In either case, this would result in there being fewer than 250 independent marker loci. The results of the simulations used the original marker data and so should be closer to "reality." However, for two or more putative QTL out of six families, more significant associations were observed than expected either from binomial calculation or from the simulations. The probability of obtaining three families significant at P<0.05 plus one significant at P<0.01, as was observed using the real *Dothistroma* data, is very small indeed (P=0.0000176).

Table 2 Mean number of putative associations in 1,000 analyses of six *Pinus radiata* full-sib families using observed genotypic data from 250 markers and randomly-generated *Dothistroma* needle blight data, where ~*N*(−0.13889, 0.416678). Expected values are calculated from Table 1, and the observation made from actual *Dothistroma* data

Number of significant families out of 6 (*n*)	Probability level (*P*)					
	0.05			0.01		
	Average from simulations	Expected	Obtained from real data	Average from simulations	Expected	Obtained from real data
0	211.57	183	202	239.39	236	236
1	35.49	58	28	10.60	14	17
2	2.63	8	18	0.01	0	0
3	0.31	1	1	<0.001	0	0
4	<0.001	0	1	<0.001	0	0
5	<0.001	0	0	<0.001	0	0
6	<0.001	0	0	<0.001	0	0

Table 3 *F*-probabilities for marker loci showing significant associations (*bold font*) with resistance to *Dothistroma* needle blight in more than one *Pinus radiata* full-sib family

Locus	Parents					
	31053 31029 Cross 1	31053 31088 Cross 2	31053 51259 Cross 3	31053 31032 Cross 4	31051 31032 Cross 6	31054 31032 Cross 7
10.63	0.70	**0.05**	0.41	–	0.18	**0.03**
14.24	–	–	–	**0.03**	**0.02**	0.83
1021a	**0.02**	**0.01**	0.35	**0.03**	0.12	0.67
1021b	**0.04**	**0.04**	0.35	**0.01**	**0.05**	0.63
2009	**0.02**	**0.01**	0.27	**0.02**	0.52	0.65
2020	**0.03**	0.28	0.56	**0.05**	0.69	0.67
Pr005	–	–	0.24	0.32	**0.05**	**0.05**

QTL detection

A list of significance levels for QTLs in each of the detection families is presented in Table 3. There were seven markers significant at the 1% or 5% levels in at least two families (*10.63*, *14.24a*, *1021a*, *1021b*, *2009*, *2020a,* and *Pr005*). One of these markers, *1021b*, was significant in three families and highly significant in a fourth.

With codominant markers, it is possible to suggest a model for the inheritance of QTL alleles and to predict which parent (and also which grandparent) has contributed the QTL effect. Assuming the simplest model possible, these are presented for the markers associated with resistance in the detection families (Table 4). Where the marker is only segregating on one side of the pedigree, the origin of the QTL effect is clear, e.g., for locus *10.63* the effect is obviously inherited through the maternal parent in cross 2 and through the paternal parent in cross 7.

With fully informative marker loci, it is possible to study the effects of QTL loci in both parents. One of the QTLs was identified by three closely linked marker loci (r=0): *1021a*, *1021b*, and *2009*. Marker *1021b* was significant in crosses 1, 2, and 6, and highly significant in cross 4. Marker locus *2009* is fully informative in cross 1 (15 × 23) and cross 4 (15 × 14). ANOVAs for this locus in these two crosses were performed partitioning out

Table 4 Phase relationships and origin of QTL effects for loci associated with resistance to Dothistroma needle blight in six *Pinus radiata* full-sib families. The letter "M" precedes marker alleles and "R" precedes resistance alleles. Lower numbered R alleles are more resistant. Marker genotypes are oriented with respect to grandparents, maternal grandparent allele on *top*, paternal grandparent allele on *bottom*. More than four alleles may be detected because there were seven different parents involved in the crosses

Locus	Cross	Maternal genotype	Paternal genotype	Origin of QTL effects
10.63a	2	M1 R1 M3 R2	M1 R2 M1 R2	31053
	7	M1 R2 M1 R2	M1 R2 M3 R1	31032
14.24	4	M2 R2 M2 R2	M2 R2 M1 R1	31032
	6	M1 R2 M1 R2	M2 R2 M1 R1	31032
1021a	1	M4 R4 M2 R3	M1 R2 M4 R1	31029
	2	M4 R4 M2 R3	M2 R2 M2 R2	31053
	4	M4 R4 M2 R3	M4 R2 M2 R1	31032
1021b	1	M1 R4 M2 R3	M1 R1 M2 R2	31029
	2	M1 R4 M2 R3	M2 R2 M2 R2	31053
	4	M1 R4 M2 R3	M1 R2 M2 R1	31032
	6	M1 R1 M2 R2	M1 R2 M2 R1	31032, 31051
2009	1	M5 R4 M1 R3	M2 R2 M3 R1	31029
	2	M5 R4 M1 R3	M4 R2 M4 R2	31053
	4	M5 R4 M1 R3	M1 R1 M4 R2	31032
2020	1	M3 R2 M1 R1	M2 R2 M1 R1	31029, 31053
	4	M3 R2 M1 R1	M2 R2 M2 R2	31053
Pr005	6	M9 R2 M9 R2	M4 R1 M2 R2	31032
	7	M9 R3 M9 R4	M4 R1 M2 R2	31032, 31054

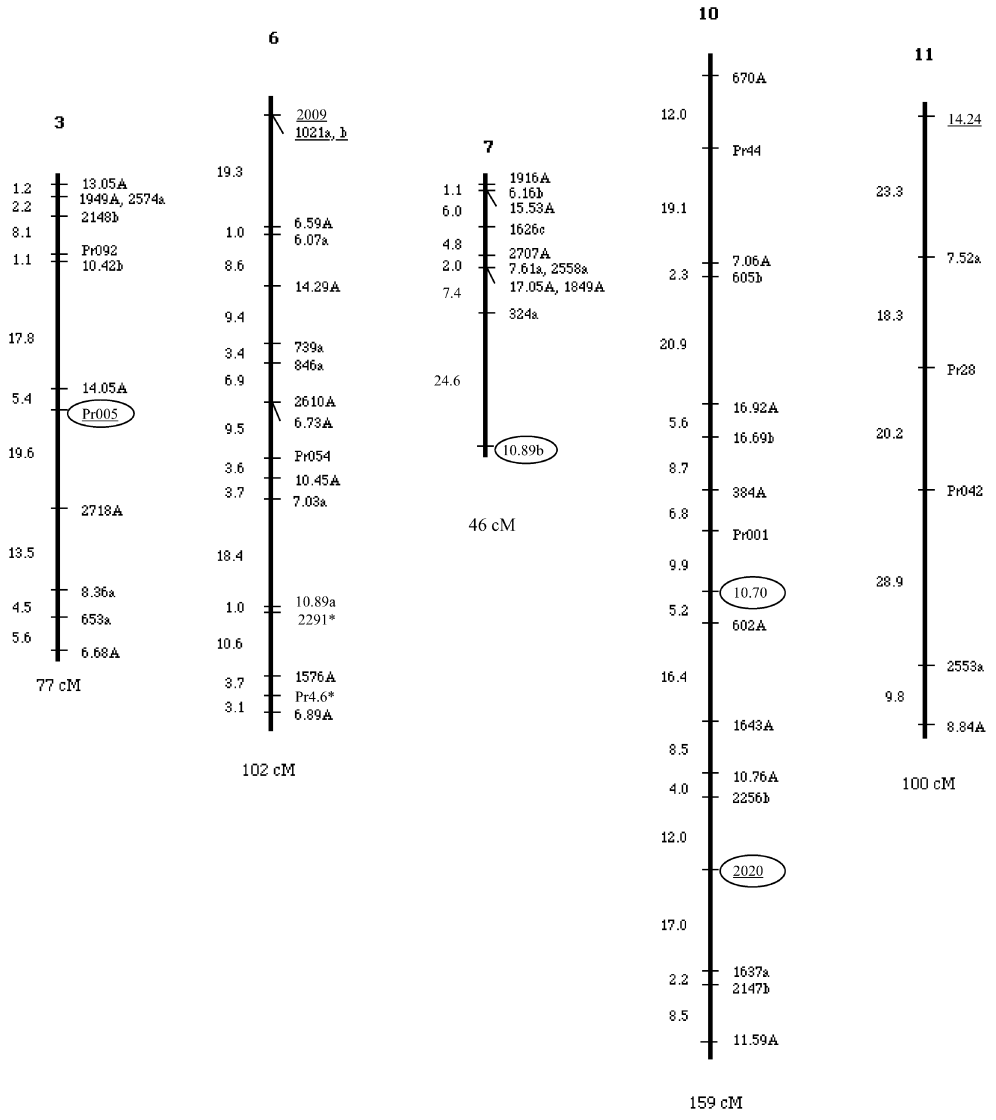

Fig. 2 A partial linkage map for *P. radiata* showing linkage groups where QTLs for resistance to Dothistroma needle blight were verified among families (*underlined*) and/or among environments using clonally replicated progeny (*circled*). Markers are shown on the *right side* and genetic distances in centiMorgans are shown on the *left*

maternal, paternal, and maternal × paternal interaction. In both cases, the male effect was highly significant (data not shown); however, the males were not the same (31029 and 31032). There is apparently also a QTL effect inherited from the maternal parent, 31053, because in cross 2 where the other parent is homozygous for the marker locus there is still a significant QTL effect. A similar relationship can be deduced for alleles segregating at *1021a* and *1021b*.

RFLP and microsatellite markers had been previously mapped in a larger number of progeny from 31053 × 31032 (Devey et al. 1996, 1999). Using this map, it was possible to determine the linkage group and position of all markers showing an association with resistance, except for *10.63*, which was not segregating in this cross. Based on map position, it appears that at least four QTL positions were identified and verified among families (Fig. 2). Three of the markers are on separate linkage groups, *Pr005, 2020A,* and *14.24* on linkage groups 3, 10, and 11, respectively. The group of three closely linked markers on linkage group 6 (*1021a, 1021b*, and *2009*) are apparently detecting the same QTL. Percent variance accounted for would be over-estimated and was not calculated for QTLs verified in the detection families due to the relatively small number of progeny per cross (Beavis 1998; Melchinger et al. 1998).

Clonal verification

Additional progeny from cross 4 were clonally replicated and planted near Bondo, NSW and Myrtleford, VIC. Both locations were in areas which in most years would have considerable *Dothistroma* infection. However, due to a sequence of years with very dry environmental conditions in late spring and early summer, which are unfavorable for expression of disease symptoms, it was not possible to make assessments for resistance at either trial site until the trees were 5- or 6-years old. Both trials were evaluated within about 6 weeks of each other; however, the

Table 5 Clonal verification of QTLs for resistance to *Dothistroma* needle blight in *Pinus radiata* full-sib family 31053×31032. Two locations were used for evaluations of resistance: Bondo, NSW (B) and Myrtleford, VIC (M). Data adjusted for rows, columns, and replicates

Linkage group	Locus	Parental genotypes	Location	*F*-probability[a]			% Variation explained	Genotypic class means[b]			
				Maternal (31053)	Paternal (31032)	Both					
								34	**39**	**44**	**49**
3	***Pr005***	34×49	B	0.359	0.384	0.028*	2.37	2.864	2.748	2.73	2.962
			M	0.114	0.023*	0.036*	2.15	2.819	2.702	2.839	2.79
								22	**24**	**44**	
6	***1021a***	24×24	B	0.336	0.336	0.336	0.08	2.906	2.792	2.808	
			M	0.431	0.431	0.431	0	2.81	2.792	2.741	
								11	**12**	**22**	
6	***1021b***	12×12	B	0.742	0.742	0.742	0	2.833	2.807	2.897	
			M	0.143	0.143	0.143	1.59	2.695	2.768	2.836	
								11	**14**	**15**	**45**
6	***2009***	15×14	B	0.785	0.268	0.520	0	2.897	2.769	2.825	2.816
			M	0.715	0.079	0.364	0.07	2.802	2.746	2.817	2.75
								11	**12**		
6	***2291***	12×11	B	0.144	–	0.144	0.42	2.78	2.87		
			M	0.313	–	0.313	0.01	2.763	2.799		
								11	**12**	**13**	**23**
6	***Pr4.6***	12×13	B	0.334	0.051	0.175	0.77	2.876	2.905	2.72	2.811
			M	0.789	0.649	0.848	0	2.786	2.769	2.774	2.811
								19	**99**		
7	***10.89***	99×19	B	–	0.016*	0.016*	1.76	2.903	2.755		
			M	–	0.824	0.824	0	2.78	2.787		
								12	**19**		
10	***10.70***	11×29	B	–	0.000**	0.000**	4.80	2.936	2.706		
			M	–	0.991	0.991	0	2.783	2.782		
								12	**23**		
10	***2020***	13×22	B	0.001**	–	0.001**	3.53	2.728	2.926		
			M	0.929	–	0.929	0	2.781	2.784		
								12	**22**		
11	***14.24***	22×12	B	–	0.688	0.688	0	2.818	2.842		
			M	–	0.120	0.120	0.53	2.81	2.755		

[a] Analyses of variance were conducted using separate male, female and combined male/female marker segregation data
[b] Numbers in *bold font* represent progeny genotypic classes obtained from the mating in column 3
* $P<0.05$, ** $P<0.01$

optimum time for making these assessments may have been missed at Myrtleford. In contrast to the Bondo site, at Myrtleford it was difficult to determine with complete certainty what symptoms were caused by *Dothistroma* and what may have been caused by secondary infection by other pathogens or natural senescence due to commencement of canopy closure.

Ten markers were tested for quantitative trait associations in the clonally replicated populations. This included all of the markers listed in Table 3, except for 10.63 which was not segregating in this cross. Also included were four additional markers (*10.70, 10.89, 2291,* and *Pr4.6*) which were highly significant only in the cross 4 detection family. Of the ten markers, *10.70*, *10.89*, *2020A*, and *Pr005* were significantly associated with resistance in the clonally replicated populations (Table 5). *Pr005* was significant in both Bondo and Myrtleford populations. *Pr005* and *2020A* were verified both among detection families and between seedlings and clones.

ANOVAs for the fully informative marker Pr005 could test for maternal, paternal and maternal × paternal interaction effects. At the Bondo location, the effects for both parents were non-significant; however, the interaction was highly significant ($P<0.01$). At the Myrtleford location, the QTL effect for the paternal parent was significant and the interaction was non-significant (data not shown).

Pr005 and *10.89b* were located on linkage groups 3 and 7, respectively; and *10.70* and *2020A* were both located on linkage group 10 (Fig. 2). The four QTLs each explained between 1.76% and 4.80% of the phenotypic variation, and if they are all independent, the total percent variation accounted for is 12.5%.

Discussion

QTLs for resistance to Dothistroma needle blight were identified using six related full-sib families of radiata

pine. Based on the probabilities of observing more than one putative association for a particular marker, it was determined that one form of QTL verification could be obtained by comparisons among detection families. Because the families were related, it was possible to predict which parent was contributing the QTL effect and to confirm this in related families. Seven markers, representing at least four QTLs, were verified in this way. A second form of verification was obtained using clonally replicated progeny from a cross of the two common parents, 31053 × 31032. In a subset of markers tested in the clonal populations, four QTLs were verified, and two of these had also been verified among the detection families.

Where a large number of correlated tests are performed an empirical distribution is needed to determine a threshold level of significance (Churchill and Doerge 1994). In the present study, the analyses were repeated many times, each time with a different set of randomly generated phenotypic data. The results showed that more associations between markers and resistance were observed than would be expected on the basis of chance for two or more families. It was therefore assumed that markers significant at the 5% level of probability in two or more families were associated with a "real" QTL effect. Further testing using the clonal populations focused on those markers that had been identified in this way.

A criticism of this study may be that the family size was inadequate to detect small effect QTLs (Beavis 1998). This is often the case in forestry where the cost and time involved in obtaining more appropriate pedigrees is prohibitive. In this study, it would be difficult to determine how many QTLs were missed (type II error). Numerous QTLs were detected among individual families; however, only four of these were detected in two or more families. A limited number of progeny per family may have been offset to some extent by an increase in the number of parents. Muranty (1996) reported that the number of parents affects the power of QTL detection. This is because the total variance in a population attributable to a QTL is better sampled with more than two parents than with only two parents. Kumar et al. (2000) reported that with a given experimental size, of say 1,000 progeny, greater power to detect QTLs was obtained with fewer large families than with many small families. Both the Muranty (1996) and Kumar et al (2000) studies were simulations and the QTLs were assumed to be real; no comparisons were made among families to determine if the same QTL was detected in multiple families.

Clonal replication and testing has the effect of increasing the heritability of individual genotypes and may be an alternative to large progeny numbers in some instances to improve the accuracy of phenotypic assessment for low heritability traits (Bradshaw and Forster 1992). As well as being a confirmation among environments, the clonal populations could be considered a confirmation of QTLs for resistance to *Dothistroma* in seedlings versus clones, although the seedlings in the cross 4 detection family were not the same individuals as those that were clonally replicated. A difference in maturation age could account for some of the differences observed in QTL expression among the detection families and clonal populations. Ades and Simpson (1990) reported a lower mean *Dothistroma* infection for *P. radiata* cuttings versus seedlings, and attributed this to a greater maturation age of the cuttings.

We have assumed that the QTL position represented by *1021a, 1021b* and *2009* on linkage group 6 was real; however, it was not confirmed in the clonal material. In the detection families, this QTL was significant in crosses 1, 2, and 6 and highly significant in cross 4. From ANOVAs involving the fully informative marker *2009*, it was determined that there were two different males contributing the QTL effect in crosses 1 and 4 (Table 5). Also, the female effect from 31053, which was common to crosses 1, 2, and 4, was significant in cross 2 but not in crosses 1 and 4. It appears that there are at least four QTL alleles segregating at this locus, or alternatively, there may be more than one QTL locus segregating in this region.

A similar result was obtained for *Pr005* which appeared to have a different expression at the Bondo and Myrtleford locations (data not shown). An interaction of male and female alleles at Bondo is an indication of dominance, and there appears to be a dominance × environment interaction at this locus. An understanding of the stability of QTL expression in different environments and in seedlings versus clones is important for marker-aided selection. Clearly, the QTLs represented by *1021b* and *Pr005* will not be as useful in a breeding program for disease resistance as would markers showing a consistent additive effect.

From these results, it should now be possible to directly identify individuals carrying QTLs for resistance to Dothistroma needle blight in radiata pine. Immediate gains in resistance can be achieved by clonal propagation of seedlings selected within families. Further investigation is needed in additional full-sib families using these or other closely linked markers.

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