Genetic Variation in Resistance to Pine Pitch Canker and Western Gall Rust in Monterey Pine (*Pinus radiata* D. Don): Results From a Three-Country Collaborative Field Trial

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In 1998, Australia, Chile, and New Zealand agreed to work together in a program designed to test their elite breeding lines and to test for the genetics of resistance to pitch canker (causative organism *Fusarium circinatum*). Pitch canker was first discovered in the United States in 1946 and in California in 1986. The first discoveries in California were in ornamental plantings, and the disease was first found in native stands of Monterey pine (*Pinus radiata* D. Don) in 1992. By 1997, pitch canker was found in 22 counties in California, and the Board of Forestry established a "Zone of Infestation." Early estimates of resistance levels were as low as 3 percent. Pitch canker is also found in Spain, South Africa, Chile, Haiti, Mexico, Portugal, and Chile. The pathogen has not been found in Australia or New Zealand, and its introduction could result in an economic disaster to the respective softwood forest industries. Due to the widespread use of Monterey pine around the world (table 1), there was interest in assessing the risk of forest plantations outside the United States for pitch canker.

Table 1—Estimated land area used for Monterey pine plantations worldwide in 1999 (adapted from Balocchi, et al. 1999)

Country	Area (000 ha)	Production (1000m^3/yr)
Chile	1 380	18 548
Australia	1 338	17 000
New Zealand	642	10 400
Spain	237	2 000
South Africa	66	486
Other	100	NA
Totals	3 763	48 434

The first step was a conference (called IMPACT Monterey) held at Monterey, California to bring together present knowledge of the disease, its epidemiology, known vectors, as well as host responses. Proceedings were published by the Australia Commonwealth Scientific and Industrial Research Organization (CSIRO) (Devey et al. 1999). The planned effort included collaboration in California for greenhouse trials and field trials of greenhouse screened lines.

Phase 1 of the IMPACT Project, a greenhouse trial, was conducted at Pebble Beach, California, comparing the responses of more than 500 open- or control-pollinated families to inoculation by *F. circinatum*. Results using a t-test showed there were significant differences between families for the length of the lesion developed 6 weeks following inoculation (Matheson et al. 2006). Estimates of heritability, using the statistical software package ASReml, in the different populations are presented in table 2. There were also significant differences between male parents, but apparently not between female parents (table 3). The next step was to test the

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same families in the field to see if the family rankings would match those obtained in the greenhouse.

Table 2—Individual tree heritability in the analysis of lesion length (adapted from Matheson et al. 2006)

Population	Heritability	SE (Heritability)	Residual variance
Chile CP	0.34	0.06	192
Año Nuevo	0.78	0.29	206
Monterey	0.46	0.26	169
Australian crosses	0.34	0.05	162
NZ OP	0.49	0.08	158

Table 3—Heritability estimates for lesion length separated by parent sex (adapted from Matheson et al. 2006)

Term	Variance component	Standard error of variance component	Heritability estimate	Standard error of heritability estimate
Female	1.9	3.7	0.04	0.08
Male	16.8	6	0.38	0.12
Combined female X male	11.4	5.4	0.21	0.07

In 2005, Phase 2 of the IMPACT Project, a field trial was set up at Davenport, California at the Swanton Pacific Ranch, owned by California Polytechnic State University. A total of 264 seedlots were available to test disease scores in the same environment as a disease-affected natural stand of the species. Seedlots from Chile were selected from among those tested in Phase 1, so as to cover the range of response; those from New Zealand were selected from among the better performing families in Phase 1. New seedlots were included from Australia to include reciprocal pairs of controlled pollinations, but also included seedlots with a range of performance from Phase 1. The trial was designed to be removed before pollen from the trial would materially affect the gene pool of the native stand nearby. Environmental factors, including wet areas in the planting site and deer damage, were analyzed to eliminate those factors from the analysis. The analysis of the deer damage showed that the damage was associated with replicate (location in the planting site) and height of the trees at the time they were damaged. High mortality following the planting in 2005 resulted in the mapped wet areas.

The field trial was assessed annually for disease symptoms until the final assessment in February 2011, after which the trial was removed. Apparent quite early in the life of the trial were galls produced by the western gall rust (causative organism *Endocronartium harknessii*), acquired both at the Institute of Forest Genetics at Placerville, where many of the trial seedlings overwintered, as well as at Swanton, following planting. Other disease symptoms included those caused by *Diplodia pinea*, particularly in the 2009 assessment. Pitch canker was confirmed present in the trial in 2009, and became more severe in 2010 and 2011.

Western gall rust findings were analyzed to look for differences among families (seedlots) in resistance. A mixed model, treating seedlots and replicates as fixed rows and columns (random), was used to obtain an estimate of the statistical significance of the differences between families. The differences between seedlots using a logarithmic transformation are highly statistically significant ($P<0.001$). The fact that the difference between families is so strong suggests that the heritability will be high. A proper genetic analysis is not done yet, so no estimate for the heritability is available at this time.

Diplodia blight outbreaks in Monterey pine in the United States have not been considered significant, but have caused significant damage in Monterey pine plantations in the Southern Hemisphere (Peterson 1981). The native population has extensive pycnidia presence, but no other

symptoms. The plantation suffered extensive tip mortality, resulting in stunting, excessive branching and forked tops. Little, if any, mortality was recorded due to Diplodia blight. In this experiment, symptomatic trees were found to have a significantly clustered distribution, which is consistent with rain splash being the primary mechanism of spore dispersal in *D. pinea*.

Preliminary analysis of the pitch canker data used only the blocking structures (replicates, rows, and columns) provided for in the original design and included the tree height as a covariate. Replicates were treated as fixed effects, the rows and columns as random effects. A binomial model with a logistic link function rather than models using normal distribution suggests the heritability is 15.21 percent ± 9.44 percent (or 0.1521 ± 0.0944). A fitted individual-tree mixed model in ASReml was utilized to get the BLUP (Best Linear Unbiased Predicted) breeding values for the parents of the trees involved in the trial. The correlation with the greenhouse results is 0.284, using the Chilean and New Zealand families only.

Some potential explanations of poor correlation with greenhouse results are as follows: 1) Presence-absence data collected in the field versus measured values for lesion lengths in the greenhouse; 2) Artificial inoculation in the greenhouse versus natural infection from vectors or wounds in the field trial; 3) Escape of susceptible individuals in the field trial; and 4) Difficulty in accurate assessment of large trees in the field.

Work on a complete analysis of the heritability of resistance to western gall rust and pitch canker is continuing.

Acknowledgments

Funding for this study came from The Agricultural Research Initiative, California State University; CSIRO, Australia; Scion, New Zealand; and Bioforest SA, Chile. Thanks to Swanton Pacific Ranch, California Polytechnic Corporation for land use, equipment, and personnel.

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