Resistance

Maturation-Related Resistance of *Pinus radiata* to Western Gall Rust

D. Zagory and W. J. Libby

Graduate research assistant, Department of Plant Pathology, and professor, Departments of Genetics and of Forestry & Resource Management, University of California, Berkeley 94720. Present address of senior author: Department of Environmental Horticulture, University of California, Davis 95616.

ABSTRACT

Zagory, D., and Libby, W. J. 1985. Maturation-related resistance of *Pinus radiata* to western gall rust. Phytopathology 75:1443-1447.

Pinus radiata (common name, radiata pine) propagated from juvenile (hedged) and more mature (nonhedged) donor plants of the same set of clones were compared to determine the effects of maturation on resistance to western gall rust, which is caused by the fungus, *Endocronartium harknessii*. The more mature plants had significantly fewer stem and branch galls than did the more juvenile ones during 2 yr following exposure to heavy natural inoculum. Clonal heritabilities were generally high and interactions between clones and maturation states were near zero. The use of selected clonal planting material and the manipulation of the maturation state of those clones may be useful in reducing the impact of western gall rust on *P. radiata*.

Additional key words: clonal forestry, disease resistance, ontogenetic ageing, *Peridermium*.

The use of stecklings (plantable rooted cuttings) in forest plantations offers attractive possibilities for both practical forestry and forest research. Some advantages and disadvantages of clonal forestry have been discussed elsewhere (2,8,9,17).

Ontogenetic ageing in plants refers to the process of moving through different stages of development, from seed germination to senescence (3). The change, in perennial plants, from juvenile to mature characteristics is an aspect of ontogenetic ageing. Generally, plants don't age ontogenetically as a whole but rather from the base to the top and from the inner to the outer part (3). Thus, the lowest, first initiated, and chronologically oldest part of a tree is the most juvenile part while the most recently formed tissues are the most mature (3). Maturity in *Pinus radiata* D. Don (common name, radiata pine) is associated with a decrease in the number of cuttings that root, a decrease in the speed of rooting, and a decline in root system quality (11). Changes in disease resistance with ontogenetic ageing in radiata pine were addressed in the current research.

The hedging of radiata pine cutting donors has been shown to arrest the maturation process (1,6,10,11). After taking cuttings from hedged and nonhedged donors of the same clone, it is then possible to compare sets of stecklings that differ in maturation state but do not differ in genotype or chronological age.

Maturation-related resistance to diseases is one variable to be considered in clonal forestry. Maturation-related differences in disease resistance among forest trees have been reported. Cuttings taken from older trees of *Thuja plicata* Lamb. were more resistant to *Didymascella thujina* (Dur.) Maire than were seedlings from the same trees (15). Patton (14) compared seedlings and grafts of *Pinus strobus* L. using scions from 4-, 10-, 20-, 40-, and 80-yr-old trees. Among the grafts, he found decreasing susceptibility to *Cronartium ribicola* J. C. Fisch. ex Rabenh. with increasing age of scion, and the seedlings were the most susceptible. Resistance of *P. radiata* to *Scirrhia pini* Funk & Parker is associated with host maturity (7). However, this prior research did not include simultaneous control of both genotype and maturation state.

Western gall rust is a disease caused in hard pines by *Endocronartium harknessii* (Moore) Hiratsuka (previously named *Peridermium harknessii* Moore) (5). This disease occurs in all three mainland native populations of *P. radiata* and is serious in many plantations of that species in California. The disease is currently restricted to North America. Since it can spread from pine to pine, western gall rust poses a considerable threat to plantations of *P. radiata* in many other parts of the world (13). As part of our investigations of patterns of susceptibility to this disease, we produced clonal pairs of stecklings that were of identical genotype and chronological age, but in two different maturation states, and planted them near trees heavily infected with western gall rust. This publication reports the results of this test and its implications for clonal forestry.

MATERIALS AND METHODS

In spring 1972, 13 seeds were germinated from the New Zealand

select radiata pine full-sib family, NZ55×19, along with 15 random seeds from a bulk sample collected from 10 native trees in each of three locations in the Monterey Peninsula in California. In midsummer 1972, these greenhouse-grown seedlings were pruned (hedged) below 0.5-m height to induce production of new shoots. Within 6 wk these shoots provided an average of 15 cuttings per seedling. Thereafter, the original seedlings continued to serve as cutting donors and were repeatedly hedged at heights of less than 0.5 m.

In May 1973, four stecklings from each of 10 of the New Zealand (NZ) seedlings and from 13 of the Monterey (MM) seedlings were planted in a demonstration plantation at the University of California's Russell Reservation (latitude 37° 55′ N, longitude 122° 08′ W, elevation 245 m). The hedged seedlings were also planted adjacent to this demonstration plantation in May 1973, and continued to be maintained as hedged cutting donors. Growth of the stecklings in this 1973 demonstration plantation was not impeded by pruning or other cultural activities. All stecklings in the
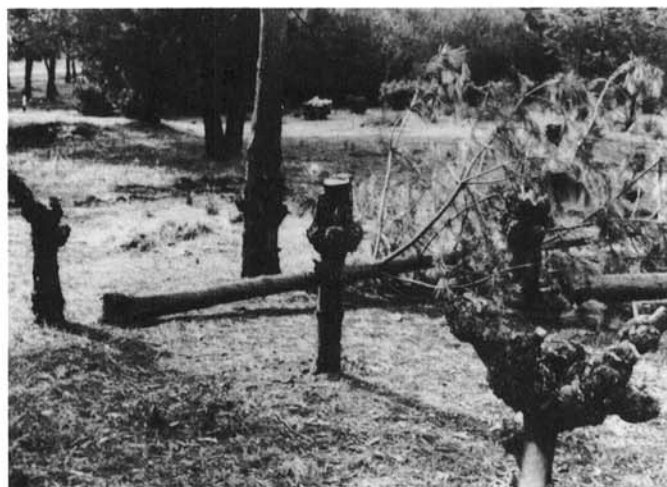

Fig. 1. Severe galling of lower stems of six *Pinus radiata* trees in the 1973 demonstration planting (photo, 1979). The tree stumps in the foreground are those of trees killed by beetle attack near the stem galls. The fourth and fifth trees were recently broken off at a stem gall by a windstorm. The sixth tree, less severely galled, was still alive in 1979.
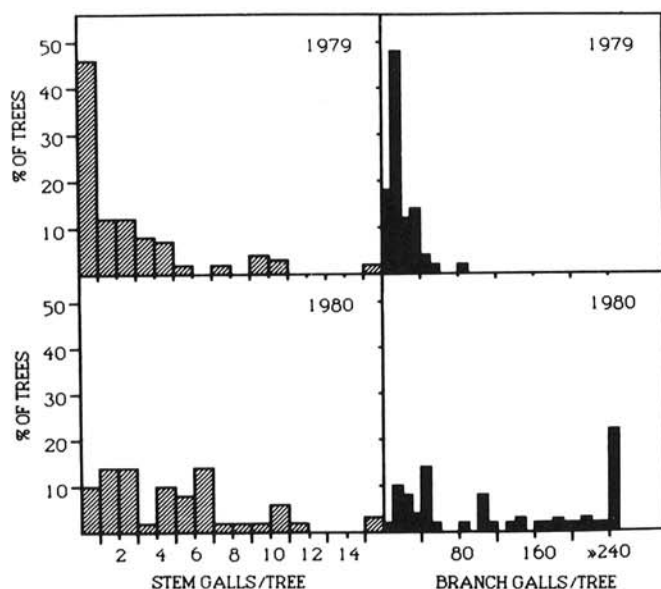

Fig. 2. Distributions of numbers of stem galls (left) and branch galls (right) per tree in the years 1979 and 1980 on stecklings of *Pinus radiata* planted in 1977 at the University of California Russell Reservation. There were 74 trees in 1979 and 70 trees in 1980.

1973 demonstration plantation developed stem and branch galls of western gall rust during the period 1973–1976 (Fig. 1).

In fall 1976, as mortality was becoming serious in the 1973 demonstration plantation, cuttings were taken from 8 NZ and 13 MM nonhedged (tree-form) stecklings at a height of approximately 2 m. Cuttings were taken the same day from the adjacent hedged seedlings. (One hedged seedling was dead and a second was able to supply only a few gall-free cuttings. Cuttings were taken from other stecklings of these two seedlings that had also been hedged at 0.5-m height since 1973 and had been maintained in 3.8-L cans containing potting soil in a greenhouse 15 km distant.) The cuttings were rooted in leach tubes by using procedures already described (10). The maturation-state differences between the resulting stecklings from hedged and nonhedged donors corresponded to the maturation that accumulated during 1.5 m of unimpeded height growth of the nonhedged donors while comparison donors had had their maturation arrested at a more juvenile state by repeated hedging at 0.5-m height (1,6,11).

In April 1977, the stecklings in leach tubes were outplanted at 2-m spacing in pairs such that one of hedge origin and another of tree (nonhedged) origin (both of the same clone) were included in each pair. Two pairs from each clone were randomly allocated to planting spots, and were naturally and rapidly infected by *E. harknessii* (primarily from inoculum produced in the adjacent 1973 demonstration plantation). All four ramets of each clone were of similar size and vigor at the time of planting.

By 1979, all stecklings in the 1977 plantation had become infected by *E. harknessii* and galls were visible. In the summer of 1979, and again in the summer of 1980, each tree in this plantation was surveyed for number of galls on the main stem, number of galls on the branches, height of the highest stem gall, and tree height. After square-root transformation of the data, analyses of variance were employed to analyze genetic and environmental components of gall-rust susceptibility and to estimate broad-sense heritabilities. In these analyses, only replicated (all four members still living) clones were included. Thus, some analyses have smaller sample sizes in 1980 than in 1979 as a few trees had died or broken between the 1979 and 1980 observations.

RESULTS

By 1980, the New Zealand stecklings, on the average, were significantly taller than the Monterey stecklings (4.5 versus 3.8 m). However, the New Zealand and Monterey stecklings did not significantly differ from each other with respect to number of branch galls, number of stem galls, or height of highest stem gall (*unpublished*). Furthermore, the patterns of differences between hedge-origin and tree-origin stecklings for these latter three characteristics were essentially identical in the New Zealand and Monterey data in both years. Therefore, we have combined the

TABLE 1. Average numbers of western gall rust galls on stems and branches of *Pinus radiata*, highest gall, and tree height in 2 yr, by steckling origin

Parameter	Steckling origin		$P(H=T)^a$
	Hedge (H)	Tree (T)	
1979			
n	37	37	
Branch galls	15.7	7.8	<0.01
Stem galls	3.2	0.9	<0.01
Highest gall (m)	0.9	0.6	<0.05
Tree height (m)	2.6	2.7	0.49
1980			
n	35	35	
Branch galls	176	95	<0.01
Stem galls	6.9	3.1	<0.01
Highest gall (m)	2.0	1.8	>0.50
Tree height (m)	3.9	4.3	0.14

[a] Paired comparisons were made with the nonparametric chi-square test except those for tree height, which were made with paired Student's *t*-test.

data from the New Zealand and Monterey clones for most of the analyses presented below.

In 1979, 2 yr after outplanting, the hedge-origin stecklings had, on average, double the number of branch galls and triple the number of stem galls as the tree-origin stecklings, both differences being statistically highly significant. The average numbers of branch galls per steckling increased over 10-fold and the average numbers of stem galls more than doubled by the following year. As

TABLE 2. Analyses of variance for the effects of clone and maturation state on numbers of stem and branch galls of western gall rust on *Pinus radiata* (radiata pine) 2 yr (1979) and 3 yr (1980) after outplanting

Source	df	MS[a]	Expected MS[b]	Estimated component	h^2
1979					
Stem galls					
Clones	15	1.9**	$\sigma_e^2 + 4\,\sigma_c^2$	$\sigma_c^2 = 0.34$	38%[b]
Maturation	1	10.4**	$\sigma_e^2 + 30\,\sigma_h^2$	$\sigma_h^2 = 0.31$	
Interaction	15	0.5	$\sigma_e^2 + 2\,\sigma_i^2$	$\sigma_i^2 = -0.03$	
Residual	32	0.6	σ_e^2	$\sigma_e^2 = 0.55$	
Branch galls					
Clones	15	6.7	$\sigma_e^2 + 4\,\sigma_c^2$	$\sigma_c^2 = 0.60$	12%
Maturation	1	21.7*	$\sigma_e^2 + 30\,\sigma_h^2$	$\sigma_h^2 = 0.54$	
Interaction	15	0.9	$\sigma_e^2 + 2\,\sigma_i^2$	$\sigma_i^2 = -1.70$	
Residual	32	4.3	σ_e^2	$\sigma_e^2 = 4.30$	
1980					
Stem galls					
Clones	14	1.6**	$\sigma_e^2 + 4\,\sigma_c^2$	$\sigma_c^2 = 0.28$	38%
Maturation	1	13.5**	$\sigma_e^2 + 30\,\sigma_h^2$	$\sigma_h^2 = 0.44$	
Interaction	14	0.7	$\sigma_e^2 + 2\,\sigma_i^2$	$\sigma_i^2 = 0.12$	
Residual	30	0.5	σ_e^2	$\sigma_e^2 = 0.45$	
Branch galls					
Clones	14	84.7**	$\sigma_e^2 + 4\,\sigma_c^2$	$\sigma_c^2 = 18.4$	62%
Maturation	1	244.8**	$\sigma_e^2 + 30\,\sigma_h^2$	$\sigma_h^2 = 7.8$	
Interaction	14	6.1	$\sigma_e^2 + 2\,\sigma_i^2$	$\sigma_i^2 = -2.5$	
Residual	30	11 2	σ_e^2	$\sigma_e^2 = 11.2$	

[a] MS = mean square. All data were square-root transformed prior to analysis. Asterisks (**) indicate F-test significance at $P < 0.01$.
[b] Broad-sense heritability = $h^2 = [$(clonal variance)/(clonal variance + residual variance)$] \times 100$.

TABLE 3. Mean numbers of stem galls and branch galls of western gall rust in 20 clones of *Pinus radiata*

Clone[a]		1979			1980	
	n	Stem galls[b]	Branch galls[c]	n	Stem galls[b]	Branch galls[c]
Ranked clones[d]						
MM10	4	2.7 hij	20	4	7.5 kl	282 z
MM3	4	2.0 hi	18	4	5.2 kl	272 z
MM8	4	2.5 hij	23	4	8.5 l	244 z
MM13	4	7.2 ij	13	4	6.7 kl	230 z
MM12	4	4.2 hij	11	4	7.7 kl	228 yz
NZ1	4	4.0 hij	20	4	7.5 kl	160 xyz
MM5	4	0.7 h	9	4	4.7 kl	149 xyz
MM1	4	1.0 h	2	4	3.2 kl	88 xyz
NZ7	4	1.2 h	9	4	3.5 kl	83 xyz
MM4	4	1.0 h	22	4	2.5 kl	73 xy
MM2	4	0.2 h	3	4	3.2 kl	57 xy
NZ6	4	1.0 h	2	4	4.0 kl	41 xy
NZ5	4	0.5 h	4	4	1.2 k	37 x
MM9	4	0.0 h	2	4	2.7 kl	24 x
NZ2	4	1.2 h	4	4	1.7 k	19 x
Unranked clones[e]						
NZ4	4	0.2	13	2	0.5	41
NZ9	2	3.5	19	2	15.5	215
NZ10	2	1.5	15	2	6.5	76
MM6	2	0.5	6	0		
MM7	2	0.0	6	2	1.5	26
MM11	2	2.5	6	2	8.5	190
All clones	74	1.8	11	70	5.4	131

[a] Clones consist of equal numbers of hedge-origin and tree-origin stecklings.
[b] Values followed by different letters are significantly different ($P < 0.05$) according to Duncan's multiple-range test (groupings are based on square-root-transformed data).
[c] Clone effect was not significant for 1979 branch galls, and thus Duncan's multiple-range test was not performed.
[d] The clones in the upper section of this table are ranked by average numbers of branch galls in 1980.
[e] These six clones are not ranked due to incomplete replication.

in 1979, the hedge-origin stecklings had significantly more branch and stem galls than did tree-origin stecklings in 1980 (Table 1).

While not significantly different, the more mature tree-origin stecklings were 4 and 10% taller than hedge-origin stecklings in 1979 and 1980, respectively. Nevertheless, the highest stem gall per tree was significantly higher on hedge-origin stecklings in 1979 and (nonsignificantly so) in 1980 (Table 1).

Variance components for clonal, maturation, clonal × maturation interaction and residual effects, and broad-sense heritability estimates from each analysis are presented for stem and branch galls in both years in Table 2. The distributions of numbers of galls are skewed toward zero and are hence not normally distributed (Fig. 2). Therefore, gall data were square-root transformed before analyses of variance were performed. Pairwise comparisons were performed by using the nonparametric chi-square test, except for comparisons of tree heights which employed paired Student's t-tests. The largest single component of the observed variation, except in the case of branch galls in 1980, was the residual variance term. Since the stecklings were all of similar size, vigor, and condition at planting, and the enumeration of galls was subject to only minor counting errors, the residual variance term is mostly due to within-plantation, microsite-associated variance. Statistically significant components of variance were associated with both clonal and maturation differences for stem galls in both 1979 and 1980 and for branch galls in 1980. The largest component of variability in branch galls in 1980 was associated with clonal differences. The interaction terms in all four analyses were near zero. This indicates that, with respect to changes in western gall rust resistance, most or all of the clones responded similarly to hedging. Heritability estimates indicate substantial proportions of genetic variability in three of the four analyses (Table 2).

A clone-by-clone analysis of stem gall and branch gall incidence (Table 3) showed that some clones were consistently more resistant than average (e.g., NZ2, MM9, and NZ5), while other clones were consistently less resistant than average (e.g., MM10, MM8, and MM13). Furthermore, stem gall numbers per clone in 1979 were significantly correlated with stem gall numbers on those clones in 1980 ($r = 0.71$**), and similarly with branch galls ($r = 0.73$**). In 1979, branch galls per clone were not significantly correlated with stem galls on those clones ($r = 0.39$) but branch galls and stem galls were significantly correlated in 1980 ($r = 0.87$**).

DISCUSSION

We began this experiment after we noticed that most western gall rust infection occurs in the lower crown or on the lower stem of radiata pine trees. We wondered if this might be due to gradients in microclimate or to gradients in the maturation state of the host tissue. Maturation state of radiata pine varies within a tree, and further, lower parts of the tree retain more juvenile forms or expressions of a number of maturation-sensitive traits than do the upper parts of the same tree (12). Thus, in this experiment, the hedge-origin stecklings were in a more juvenile maturation state than the tree-origin stecklings, even though their chronological ages were identical (6,11). Such cutting-origin differences in radiata pine's maturation state have been maintained for at least 7 yr of steckling development (1).

That differences in maturation state are associated with differences in resistance to western gall rust is demonstrated by our data on comparative gall numbers for both 1979 and 1980. The maturation-related difference in gall rust resistance is further demonstrated by the observations on the height of the highest stem gall per tree. If gall rust resistance is maturation related, it should increase with height of the tree. Since the hedge-origin stecklings are behind the tree-origin stecklings in maturation, it is expected that the zone of gall rust susceptibility would extend further up the stem of the hedge-origin trees. This expectation is supported by the data on highest stem gall (Table 1).

Since tree-origin and hedge-origin stecklings were of similar size when planted and grew at similar rates, differences in resistance to western gall rust due to differing microclimatic effects at different

tree heights can be ruled out as the sole cause of gall distribution in radiata pine stems and crowns. In this experiment, even though the differences in maturation state between hedge- and tree-origin stecklings were small, the maturation-related differences in resistance to western gall rust were pronounced with respect to both stem galls and branch galls.

Stem galls are responsible for distortion and growth loss of bole wood. Stem galls may also lead to death of the tree due to breakage or due to gall-associated beetle attack (Fig. 1). Thus, stem galls are economically important. Branch galls do not generally grow into the stem (D. Zagory, *unpublished*). The chief economic effect of branch galls is as a source of inoculum for infection of stems.

The more rapid height growth of the tree-origin stecklings, compared to that of the less mature, hedge-origin stecklings, is not consistent with previous observations by several workers (reviewed by Bolstad and Libby [1]). In the absence of infection by *E. harknessii*, early height growth of radiata pine seedlings or juvenile stecklings is typically greater than that of more mature stecklings (1,16). This suggests that gall incidence, perhaps particularly stem-gall incidence, has a deleterious effect on height growth.

The basis of maturation-related resistance to western gall rust is unknown, but could be related to differences in epicuticular wax chemistry, as Franich et al (4) have suggested with respect to changes in resistance to *S. pini*. Differences in allocation of energy to, and production of, needles and twigs between hedge-origin and tree-origin stecklings (6) could also play a role in resistance differences. Possible morphological and phenological bases for maturation-related resistance to gall rust are under continuing study at Berkeley.

This study provides the first information on genetic variation in resistance of radiata pine to western gall rust. The genetic variation demonstrated in this experiment appears to be of two kinds: genomic (between clones) and ontogenetic (between maturation states). Together they suggest logical measures to be taken in areas where radiata pine plantations are important and western gall rust is a serious disease. Relatively resistant clones may be selected, and their resistance may be increased by propagating them in a relatively advanced maturation state. Our observations in this and other experiments indicate that a late-juvenile or early adolescent maturation state may be appropriate, providing substantially increased resistance to western gall rust and acceptable tradeoffs with other characteristics also affected by changes in maturation (1).

LITERATURE CITED

1. Bolstad, P. V., and Libby, W. J. 1982. Comparisons of radiata pine cuttings of hedge and tree-form origin after seven growing seasons. Silvae Genet. 31:9-13.
2. Fielding, J. M. 1970. Trees grown from cuttings compared with trees grown from seed (*Pinus radiata*). Silvae Genet. 19:54-63.
3. Fortanier, E. J., and Jonkers, H. 1976. Juvenility and maturity of plants as influenced by their ontogenetical and physiological ageing. Acta Hortic. 56:37-43.
4. Franich, R. A., Wells, L. G., and Barnett, J. R. 1977. Variation with tree age of needle cuticle topography and stomatal structure in *Pinus radiata* D. Don. Ann. Bot. 41:621-626.
5. Hiratsuka, Y. 1969. *Endocronartium*, a new genus for autoecious pine stem rusts. Can. J. Bot. 47:1493-1495.
6. Hood, J. V., and Libby, W. J. 1979. Continuing effects of maturation state in radiata pine and a general maturation model. Pages 220-232 in: Propagation of Higher Plants Through Tissue Culture: A Bridge Between Research and Application. Proc. Conf. 7804111. K. W. Hughes, R. Henke, and M. Constantin, eds. U.S. Department of Energy, Washington, DC.
7. Ivory, M. H. 1972. Infection of *Pinus radiata* foliage by *Scirrhia pini*. Trans. Br. Mycol. Soc. 59:365-375.
8. Libby, W. J. 1974. The use of vegetative propagules in forest genetics and tree improvement. N.Z. J. For. Sci. 4:440-447.
9. Libby, W. J. 1977. Rooted cuttings in production forests. Pages 13-19 in: Proc. 14th Southern Forest Tree Improvement Conference. 14-16 June 1977, Gainesville, FL. Southern Forest Tree Improvement Committee Publ. 36.

10. Libby, W. J., Brown, A. G., and Fielding, J. M. 1972. Effects of hedging radiata pine on production, rooting and early growth of cuttings. N.Z. J. For. Sci. 2:263-283.

11. Libby, W. J., and Hood, J. V. 1976. Juvenility in hedged radiata pine. Acta Hortic. 56:91-98.

12. Olesen, P. O. 1978. On cyclophysis and topophysis. Silvae Genet. 27:173-178.

13. Parmeter, J. R., and Newhook, J. V. 1967. New Zealand *Pinus radiata* is susceptible to western gall rust disease. N.Z. J. For. 12:200-201.

14. Patton, R. F. 1961. The effect of age upon susceptibility of eastern white pine to infection by *Cronartium ribicola*. Phytopathology 51:429-434.

15. Soegaard, B. 1956. Leaf blight resistance in *Thuja*: Experiments on resistance to attack by *Didymascella thujina* (Dur.) Maire (*Keithia thujina*) on *Thuja plicata* Lamb. Pages 30-48 in K. Vet. Landbohojsk. Arsskr., Copenhagen.

16. Sweet, G. B. 1973. The effect of maturation on the growth and form of vegetative propagules of radiata pine. N.Z. J. For. Sci. 3:191-210.

17. Thulin, I. J., and Faulds, T. 1968. The use of cuttings in the breeding and afforestation of *Pinus radiata*. N.Z. J. For. 13:66-77.