EARLY DIFFERENTIAL SUSCEPTIBILITY OF JUVENILE SEEDLINGS AND MORE MATURE STECKLINGS* OF PINUS RADIATA TO DOTHISTROMA PINI

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ABSTRACT

The relationship between maturation state of **Pinus radiata** D. Don and its early resistance to **Dothistroma pini** Hulbary var. **linearis** infection (measured as number of needle blight bands) was examined. Two-year-old seedlings, grown in the field, were found to have much higher levels of infection than related stecklings of a more advanced maturation state. By the end of the second season of growth, seedlings were almost completely defoliated as a result of the infection. There was no evidence that hedging **P. radiata** trees at different heights up to 4 m provided cuttings with any differences in susceptibility to infection. Seedlings from the Guadalupe population showed a lower level of infection than seedlings of the other populations tested.

INTRODUCTION

Dothistroma needle blight (or red band needle blight) caused by the fungus *Dothistroma pini* is a disease of considerable economic importance in some countries of the Southern Hemisphere where *Pinus radiata* has been planted extensively (Peterson & Graham 1974). In the Western United States Dothistroma needle blight is also responsible for losses of *P. radiata* and other pine species in nurseries and in demonstration and experimental plantations (Dubin & Staley 1966; Cobb & Libby 1968; F. W. Cobb pers. comm.; Peterson 1967, 1975; Peterson & Harvey 1976; Peterson & Walla 1978).

The fungus causes defoliation, usually leaving green needles only at the tips of the branches. Early signs of the infection are yellow and tan spots (and/or bands) on the needles, which may gradually turn brown and, later, red. At a later stage of infection, needles develop necrosis. The needles then die back from the tips, and eventually are shed.

Variable levels of resistance to the fungus have been found in experimental and commercial plantations of *P. radiata* in several parts of the world, and these differences have been regarded mostly as genetic in origin (Ivory & Paterson 1969; Wilcox 1982).

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* Established rooted cuttings

There is also some evidence that *P. radiata* trees develop resistance to the fungus gradually as they mature (Ivory 1972; Garcia & Kummerow 1970), but the mechanism is not yet fully understood. It has been suggested that protection against infection is provided by the presence of wax occlusions of the stomatal antechamber, which is a characteristic of more mature trees (Franich *et al.* 1977). Through clonal forestry it should be possible to select for resistance to *D. pini,* not only by selection of genotype, but also by selection of an appropriate maturation state of the stecklings (established rooted cuttings).

Three varieties of the fungus have been identified as causing the disease in *P. radiata.* While *D. pini* var. *pini* and var. *keniensis* are the disease organisms in the Southern Hemisphere and because of their economic importance have received most attention, *D. pini* var. *linearis* is responsible for the disease in the Western United States (F. W. Cobb pers. comm.; Peterson & Graham 1974). Although disease symptoms of the varieties appear very similar, there may be differences in their pathology.

This study was designed to investigate the relationship between maturation state of *P. radiata* and its resistance to *D. pini* var. *linearis* infection. Trees grown from seedlings (the most juvenile origin) were compared with trees grown from cuttings obtained from hedged trees. Hood & Libby (1978) reported that continually hedging *P. radiata* trees stops or slows the rate of maturation. Thus, if the differences in growth of cuttings taken from hedge and tree donors, reported by Bolstad & Libby (1982), were due to differences in maturation state, it should be possible to obtain trees of different maturation states by hedging them at different heights. To test whether cuttings of different maturation states, as evidenced by differences in *D. pini* var. *linearis* infection, could be obtained from trees hedged at different heights, comparisons were made between trees grown from cuttings obtained from four heights of hedging.

MATERIALS AND METHODS

Cuttings were obtained from two clones of each of three native *P. radiata* populations (Guadalupe Island, Ano Nuevo, and Monterey), and from two clones from New Zealand select families. The stock plantation from which the cuttings were obtained was established in May 1973 as newly rooted cuttings from ortets planted in 1967. Each clone was represented by four ramets of different heights (0.5 m, 1 m, 2 m, and 4 m) developed by periodic hedging after the tree had attained the desired height; the lowest height tree was expected to be the most juvenile. Each cutting donor provided 20 cuttings giving a total of 640 cuttings.

Cuttings were taken in October 1980 from the top of the hedges. They were trimmed to approximately 9–10 cm in length, and then immersed in a benlate* solution of 0.3 g/litre of water for 30 min. The base of the cutting was then freshly cut and dipped into an indole-butyric acid solution of 4000 ppm in 95% ethyl alcohol. The cuttings were inserted into pre-formed depressions in the rooting medium. All cuttings were rooted in a greenhouse under intermittent mist and with a 16-h photoperiod. The cuttings rooted between January and July 1981.

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* Active ingredient Benomyl 50%

Seeds were obtained from the same three native populations as the cutting donors, and from two New Zealand select families (55 × 121 and 19 × 55). The seeds were sown in July 1981. It was intended that this would be later than the rooting of cuttings so that both stecklings and seedlings would be of a similar size at the time of outplanting.

Seedlings and stecklings received the same hardening off and fertiliser treatments prior to outplanting.

The trees were planted on 9 March 1982 in the Jackson Demonstration State Forest, Parlin Fork Camp (17 miles east of Fort Bragg, California). A total of 225 trees were planted at 3 × 3-m spacing. These were arranged in rows of five trees, each row composed of one clone. Since two clones of each population were included in the trial, each clone was replicated a minimum of five times. Within a row, the five trees usually included stecklings from four different height origins together with a related seedling. This was not always possible because poor rooting and early mortality meant there were insufficient numbers of rooted cuttings from some height origins. Replacements by extra cuttings of other height origins of the same clone totalled 25. This had little effect on the analysis since total numbers of trees of each height origin were almost equal.

The source of *D. pini* var. *linearis* infection was from some heavily infected old *P. radiata* trees surrounding the plantation.

Data were collected 1 year from planting, on 11 March 1983. At this time no trees showed any appreciable defoliation. Susceptibility to infection by *D. pini* was assessed by counting the total number of clearly identifiable red bands on needles on each tree.

RESULTS AND DISCUSSION

At the time of data collection 1 year after field planting, there had been 19% mortality amongst trees of cutting origin compared with 4% amongst seedling trees. This mortality was not a result of infection by *D. pini,* but was due mainly to the stress associated with soil erosion exposing roots, and to animal damage. Libby & Hood (1976) have already reported more hare damage on more mature stecklings of *P. radiata.*

Three months after planting, *D. pini* var. *linearis* infection was already visible. This was apparent from the presence of yellow flecks and yellow bands and sometimes tan or red coloured bands on the older needles. At this early stage it was already evident that the seedlings were more susceptible to infection than the stecklings. The mean number of blight bands per seedling was 28.16 compared with a mean of 5.03 bands for the stecklings. Although seedlings had a greater total number of needles than stecklings, this difference was not great enough to account for the difference in number of blight bands. A one-way analysis of variance, comparing all stecklings with all seedlings, indicated a highly significant difference ($p < 0.001$) in degree of infection between the two groups (Table 1). The much greater degree of infection of seedlings than stecklings could be attributable to the earlier state of maturation of the former. The seedlings in the present study were less than a year old at the time of planting and showed morphologically juvenile characteristics such as the typical non-sealed apical

TABLE 1—One-way analysis of variance of the number of **Dothistroma pini** bands observed in seedlings and in stecklings

Source of variation	d.f.	SS	MS	F	p
Between groups (between seedlings and stecklings)	1	17 768	17 768.7	146.5	<0.001
Within groups	187	22 678	121.2		
Total	188	40 447			

bud. In contrast to seedlings, the stecklings had an elongating shoot with unexpanded needles and scale leaves enclosing the apex, which is characteristic of adolescent (or more mature) *P. radiata* trees.

Although the data suggest that more juvenile trees are more susceptible to infection, differences in morphology or physiology between seedlings and stecklings cannot be ruled out as being the cause.

The much greater degree of infection of seedlings than stecklings supports the data of Wilcox (1982) in which cuttings were taken from free-growing trees of 7 to 40 years of age.

Data on infection were collected before the disease had developed sufficiently to cause needle loss. Among stecklings mean numbers of needle blight bands per tree were $\overline{X}_{0.5\,m} = 6.2$, $\overline{X}_{1.0\,m} = 3.7$, $\overline{X}_{2.0\,m} = 4.8$, $\overline{X}_{4.0\,m} = 5.3$. A one-way analysis of variance of the stecklings, grouped by height origin, showed no significant ($p = 0.4$) differences in degree of infection among the four origins (Table 2). A gradient of declining number of blight bands on stecklings from low to high height donors would have been expected if susceptibility to the disease declines with increasing maturation of the host. Unlike any of the other stecklings, some stecklings from the 4-m height donors had well-developed male strobili that soon shed abundant pollen, suggesting that they were at a more mature stage of development.

After data collection in March 1983, *D. pini* infection increased. Needles which had shown tan and reddish bands developed necrotic tips. The necrosis spread down the needle until it was totally brown and the needle was then cast. In the stecklings the

TABLE 2—One-way analysis of variance of the number of **Dothistroma pini** bands in stecklings from four different hedge-heights (height of original cutting donors: 0.5 m, 1 m, 2 m, 4 m)

Source of variation	d.f.	SS	MS	F	p
Among groups (among four hedge-heights)	3	111	37.3	0.6 n.s.	$= 0.4$
Within groups	142	8 350	58.8		
Total	145	8 462			

proportion of needles infected remained relatively small. However, in seedlings, heavy infection first affected the 2-year-old needles, and then the oldest of the current season's growth. By June of 1983, many of the seedlings were almost completely defoliated; only the youngest needles at the top of the tree remained alive. No stecklings showed this degree of defoliation. At this late stage of infection stecklings cast a few needles, showed a few blight bands, but had abundant green healthy needles along the stem and branches.

Although it is not possible to draw firm conclusions because of the small sample size, the data suggest possible differences between populations in susceptibility to *D. pini* var. *linearis*. Among the seedlings, the Guadalupe population showed significantly fewer ($p = 0.027$) needle blight bands than the other three populations. The mean number of blight bands per seedling was 13.66 for the Guadalupe population, 27.46 for the Monterey population, 35.37 for the Ano Nuevo population, and 35.72 for the New Zealand population.

Enough variation in susceptibility (data not shown here) was present among clones to justify a more extensive study including interpopulation hybrids, to obtain a better understanding of the basis of inheritance of resistance to *D. pini* in *P. radiata*.

CONCLUSIONS

These preliminary data suggest that use of stecklings of an adolescent or more advanced maturation state relative to young seedlings, may result in greater resistance of plantations of *P. radiata* to early infection by *D. pini* var. *linearis* since stecklings are, at least initially, more resistant than seedlings. This could significantly reduce the need for intensive spraying of young trees with copper-based fungicides. However, although the stecklings appeared to have a considerably higher degree of resistance to infection up to 1 year after planting, it is not clear from this study if this greater resistance would persist through the life of the tree. It is possible that the difference in susceptibility between seedlings and stecklings would decrease as the seedlings mature and approach the maturation level of the stecklings.

Degree of infection did not differ between stecklings from different hedge heights, suggesting that hedging at different heights did not produce shoots of different levels of maturation. Although data were not presented here, rooting percentage and rate of rooting of the cuttings did not vary significantly between the hedge origins either. However, morphological observations, such as form of the terminal shoot and presence of male strobili, suggested some differences in level of maturation existed, at least when stecklings of 4-m height origin were compared with other stecklings.

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