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Variation in susceptibility to pitch canker disease, caused by *Fusarium circinatum*, in native stands of *Pinus muricata*

D. G. Schmale III‡ and T. R. Gordon*†

Department of Plant Pathology, University of California, Davis, CA 95616, USA

Two hundred *Pinus muricata* trees, located within a native forest near Monterey, California, were inoculated with the pitch canker pathogen. Treated branches were removed 10–13 weeks following the initial inoculation, and the lengths of the lesions that developed at each of the inoculation sites were measured. Results indicated that bishop pine exhibits a wide range of variation in susceptibility to pitch canker disease. Of the trees that received only one inoculation, 27% showed almost no lesion development, indicating that they were relatively resistant to the pathogen, while others had significantly longer lesions and thus were more susceptible. Clonal propagation and seed collection from resistant individuals may offer useful strategies for disease management in the future.

Keywords: bishop pine, systemic-induced resistance

Introduction

Pitch canker disease, caused by the fungus *Fusarium circinatum* [= *Fusarium subglutinans* f. sp. *pini* (Correll *et al*., 1991)], was first recognized in California, USA, in 1986, when it was isolated from diseased *Pinus radiata* (Monterey pine or radiata pine) in Santa Cruz County (McCain *et al*., 1987). Six years later, the pathogen was recovered from native Monterey pines on the Monterey Peninsula, in Monterey County, California (Storer *et al*., 1994). The disease has since intensified on the Monterey Peninsula, where it is now also affecting native stands of *Pinus muricata* (bishop pine).

Variation in susceptibility to pitch canker has been demonstrated for Monterey pine within both native and planted stands (Storer *et al*., 1999), based on the lengths of lesions developing on branches subjected to mechanical inoculations. A study using clonal lines of Monterey pine showed the ranking of pine genotypes based on lesion length to be independent of the location where trees were grown (Gordon *et al*., 1998a). Furthermore, clones sustaining only short lesions did not develop natural infections under field conditions (Gordon *et al*., 1998a; unpublished observations). In a separate study, rankings of different pine species based on lesion length correlated well with their relative susceptibilities under field conditions (Gordon *et al*., 1998b). Consequently, lesion length provides a useful indicator of relative susceptibility to pitch canker. Observed differences in lesion development and disease severity have been attributed to genotypic differences among individual hosts (Gordon *et al*., 1998a; Storer *et al*., 1999).

Bishop pine has a far more extensive range than Monterey pine, and could serve as a host bridge to more northerly locations and to other susceptible *Pinus* species. Although bishop pine is known to be susceptible to pitch canker (Correll *et al*., 1991; Clark, 1998), there has been no systematic effort to characterize variation in susceptibility within native populations of this species. The proportion of the population that manifests some degree of resistance will provide an indication of the extent to which pitch canker is likely to develop in native stands of bishop pine. Furthermore, genetic resistance represents a resource that may ultimately prove to be useful in the management of pitch canker in California and elsewhere in the world. The present study was undertaken with the following specific objectives: (i) to characterize the variation in susceptibility to pitch canker within a native population of bishop pine; and (ii) to evaluate the consistency of this characterization through repeated inoculations of individual trees over time.

Materials and methods

Field sites

Studies were conducted within a native bishop pine forest on Huckleberry Hill (36°3520′N, 121°5534′W), on

*To whom correspondence should be addressed.

†E-mail: trgordon@ucdavis.edu

‡Current address: Department of Plant Pathology, Cornell University, Ithaca, New York 14853, USA.

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the Monterey Peninsula. The Huckleberry Hill population of bishop pine is representative of the green strain of this species. One hundred bishop pines were selected at each of two field sites (referred to hereafter as site 1 and site 2). The two sites did not differ in terms of soil type or understorey vegetation and were intended to represent two independent samplings of the bishop pine population. The 200 selected trees had regenerated naturally following a wild fire in 1987, and were estimated to be approximately 12–13 years old at the time of the first inoculation. Trees included in the study had a sufficient number of branches for several rounds of inoculation, were free of disease symptoms, and had no visible evidence of insect damage; otherwise they were selected randomly.

Table 1 The time sequence of inoculations of 200 bishop pine trees on Huckleberry Hill in Monterey County, California

		Inoculation		
Tree number	Site location	Time 1	Time 2	Time 3
1–25	Site 1	X	X	X
51–75	Site 1		X	X
26–50[a]	Site 1		X	
540–564	Site 1			X
76–100	Site 2	X	X	X
126–150	Site 2		X	X
100–125[a]	Site 2		X	
515–539	Site 2			X

[a]Branch-removal control trees.

Inoculum preparation

Aqueous spore suspensions were prepared one day before each of the field inoculations, and were maintained at 4°C overnight. Cultures of a known virulent isolate of *Fusarium circinatum*, FSP 17, were grown on plates of potato dextrose agar (PDA) for 7 days. FSP 17 was originally isolated from an infected Monterey pine on the Monterey Peninsula and is associated with vegetative compatibility group C1 (Gordon *et al*., 1996). The agar cultures were flooded with a sterilized aqueous solution of 0·5% KCl and the mycelium was disrupted by scraping the plates with a bent glass rod. The resulting fungal suspension was filtered through two layers of sterile cheesecloth, and the number of spores in the filtrate was quantified using a haemacytometer. The quantified suspensions were diluted in 0·5% KCl to a final concentration of 250 spores per 5 μL. Spore suspensions were transferred to 50-mL sterile conical tubes and transported to the field on ice. Inoculum concentration was verified and spore viability assessed by making dilutions of fresh spore suspensions and distributing 100 μL of each diluted preparation onto PDA plates. Dilutions were made at a target concentration of 50 spores per 100 μL. Diluted samples were plated out on the same day the inoculum was prepared, and the resulting colonies were counted 3 days later.

Inoculation method and lesion assessment

Inoculations were conducted on 12 July 2000 (time 1), 25 September 2000 (time 2) and 6 February 2001 (time 3) (Table 1). At each time, a single inoculation was placed on each of three branches per tree. Inoculation courts were prepared as described by Gordon *et al*. (1998b), with approximately 250 spores (in 5 μL) delivered in each inoculation. Mock inoculations were not performed because numerous studies have demonstrated that lesions do not develop following these treatments (Clark, 1998; Gordon *et al*., 1998a,b; Schmale *et al*., 1999; Storer *et al*., 1999; Bonello *et al*., 2001).

Branches were collected 10–13 weeks following inoculation, and the lesion at the site of inoculation was measured as described by Gordon *et al*. (1998b). Mean lesion lengths were calculated for each tree. Branch removal and lesion assessment took place on 23 September 2000, 5 December 2000 and 12 May 2001 (for inoculations at times 1, 2 and 3, respectively).

Host response to one inoculation

At each of times 1, 2 and 3, 50 trees (25 at each site) that had not been previously inoculated were inoculated as described above (Table 1). Lesions were assessed 10 weeks after the inoculations at times 1 and 2, and 13 weeks after the inoculations at time 3. Analysis of variance (ANOVA) was used to test for significant differences in lesion length among trees at each time interval (times 1, 2 and 3) (StatView, 1998). Median lesion lengths at the two sites were compared for each inoculation time using a non-parametric Mann–Whitney test (StatView, Version 5, 1998, SAS Institute Inc., Cary, NC, USA).

Host response to branch removal

When the time 1 lesions were assessed, three branches were removed from each of a further 50 trees that had not been previously inoculated (25 at each site). At time 2, three new branches on each of these 50 trees were inoculated (Table 1). Lesions were assessed 10 weeks following the inoculations at time 2. ANOVA was used to test for significant differences between mean lesion lengths on trees from which branches were removed and trees which had received no previous inoculations.

Host response to repeated inoculations

Each of the trees originally inoculated at time 1 or time 2 was inoculated a second time. Thus, in total, 100 trees were inoculated at two different times (Table 1). Lesions were assessed 10–13 weeks after the inoculations. The 50 trees that were originally inoculated at time 1, and again at time 2, were inoculated a third time (Table 1). Correlation coefficients between lesion lengths were calculated for each possible pair of inoculation dates (StatView, 1998).

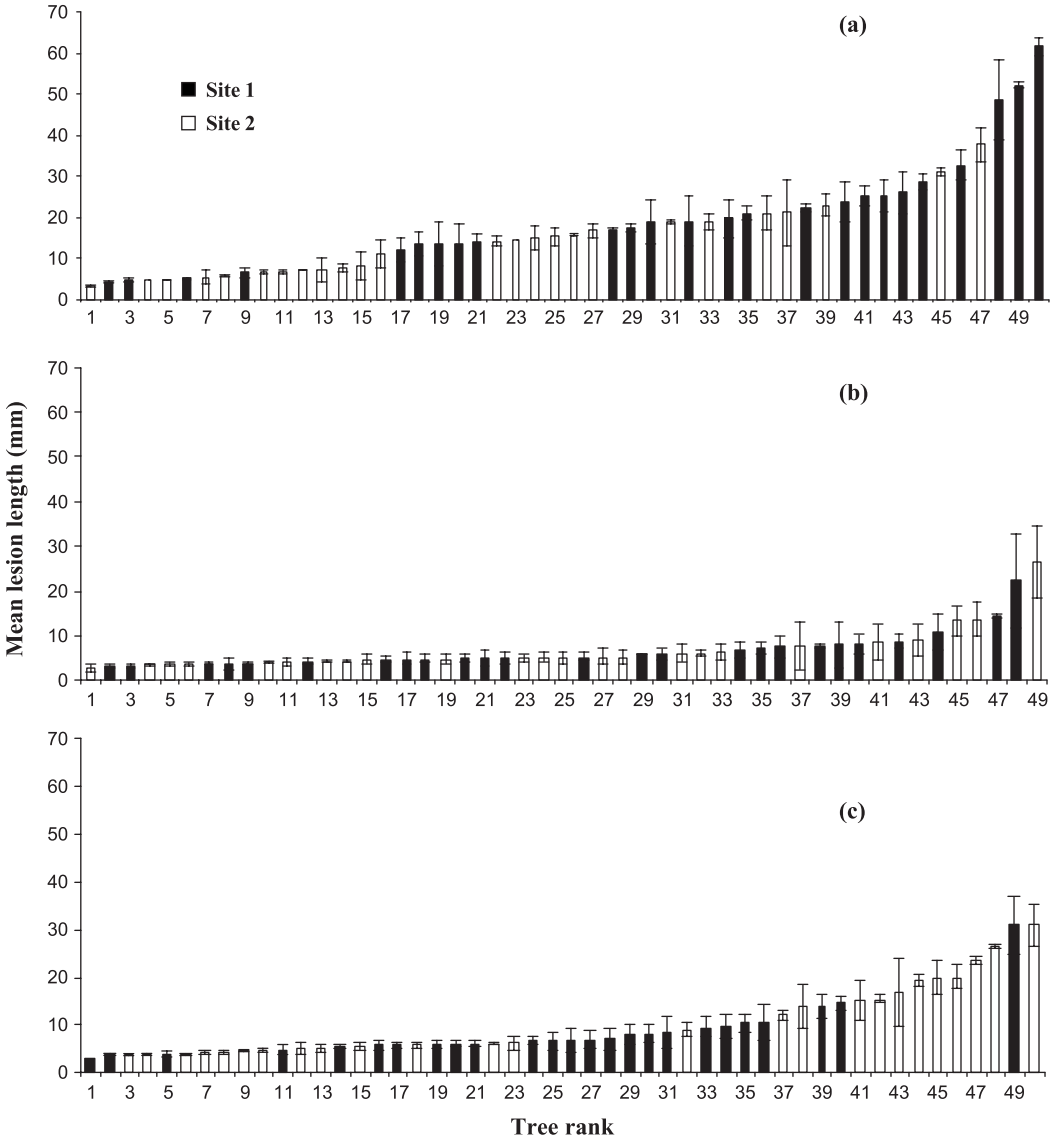

Figure 1 Ranked mean lesion lengths (mm) on bishop pine trees inoculated with *Fusarium circinatum* at time 1 (12 July 2000), time 2 (25 September 2000) and time 3 (6 February 2001). At each time, 25 trees not previously inoculated were inoculated at each of two sites. Error bars represent ± standard error. At time 1 (a), ranks of trees at site 1 were significantly greater than ranks at site 2. At time 2 (b) and time 3 (c), ranks of trees at the two sites were not significantly different.

Results

Inoculum concentration

Dilution plate counts were as follows: 55·0 ± 2·5 spores per plate for time 1, 55·3 ± 3·8 spores per plate for time 2, and 59·0 ± 3·4 spores per plate for time 3. Thus, this estimate of spore viability indicates that inoculum levels to which trees were exposed on three different dates differed by less than 10%.

Host response to one inoculation

For the 50 trees inoculated at time 1, mean lesion lengths ranged from 3·3 to 61·8 mm (Fig. 1a). The lesion lengths for trees at site 1 were significantly different from those at site 2 based on a Mann–Whitney test ($P = 0·03$). Differences among trees were significant ($P < 0·001$) at both site 1 and site 2. Among the 50 trees inoculated (for the first time) at time 2, mean lesion lengths ranged from 2·8 to 26·7 mm (Fig. 1b). For the 50 trees first inoculated at time 3, mean lesion lengths ranged from 3·0 to 31·0 mm (Fig. 1c). At both times 2 and 3, differences in lesion lengths between site 1 and site 2 were not significant (time 2, $P = 0·73$; time 3, $P = 0·70$). Analysis of the combined data for the two sites showed that differences among trees were significant at time 2 ($P < 0·001$) and at time 3 ($P < 0·001$).

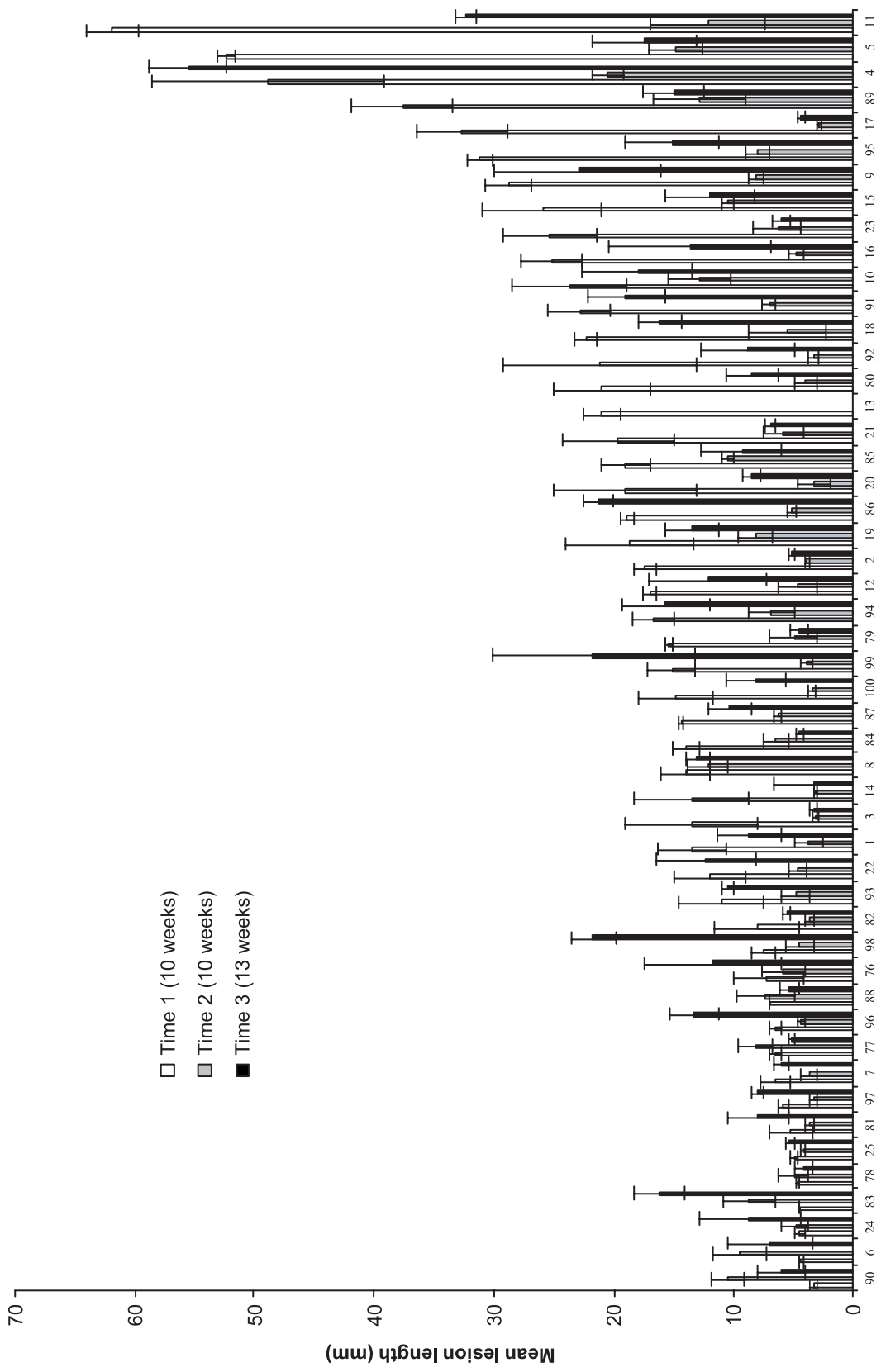

Figure 2 Response to repeated inoculations of the same 50 bishop pine trees with *Fusarium circinatum*. Error bars represent ± standard error. Correlation of inoculation dates was significant between all combinations of the three time periods. The interval between inoculation and scoring of lesions was either 10 weeks (times 1 and 2) or 13 weeks (time 3).

Host response to branch removal

Mean lesion lengths on trees from which branches were removed prior to inoculation were not significantly different from those on trees not subjected to branch removal ($P = 0{\cdot}54$).

Host response to repeated inoculations

Lesion lengths on the 50 trees that received three inoculations were significantly correlated: time 1 vs. time 2 ($R = 0{\cdot}55$, $P < 0{\cdot}001$); time 1 vs. time 3 ($R = 0{\cdot}59$, $P < 0{\cdot}001$); and time 2 vs. time 3 ($R = 0{\cdot}66$, $P < 0{\cdot}001$) (Fig. 2). Overall, mean lesion length for all 50 trees decreased from $17{\cdot}7 \pm 1{\cdot}8$ mm (mean ± standard deviation) at time 1 to $6{\cdot}5 \pm 0{\cdot}5$ mm at time 2, and increased to $11{\cdot}8 \pm 1{\cdot}3$ mm at time 3. For the 50 trees that received only two inoculations, lesion lengths were not significantly correlated over time ($R = 0{\cdot}06$, $P = 0{\cdot}70$).

Discussion

The present findings demonstrate that native bishop pines differ in their susceptibility to pitch canker disease. Lesion lengths produced by bishop pine trees in response to a single inoculation with *Fusarium circinatum* varied among trees, sites and inoculation dates. Previous work using clonal lines of Monterey pine documented that the relative susceptibility of individual genotypes was consistent over time and independent of location (Gordon *et al*., 1998a). Thus, the observed differences in lesion lengths among bishop pines in this study are likely to have a genetic basis, at least in part.

Averaged over all inoculations, 27% of the trees tested (40 out of 149) sustained mean lesion lengths of 5 mm or less, which may approximate the proportion of trees resistant to pitch canker. This estimate is based on observations in Monterey pine, which indicated that trees sustaining limited lesion development did not develop natural infections (Gordon *et al*., 1998a; unpublished observations). By analogy, something of the order of 27% of the bishop pine population from which the present sample was drawn should manifest some level of resistance to pitch canker, with some of these trees being highly resistant. Monitoring of the sampled bishop pine population over time will be required to verify this prediction.

At two of the three inoculation times, differences in mean lesion lengths between sites 1 and 2 were not significant, suggesting that both samples provided a similar representation of the relative susceptibilities of trees in this population of bishop pine. On the other hand, at time 1, the overall mean lesion length was greater for trees at site 1 than those at site 2. The reason for this difference was unknown, but may have simply been caused by chance selection of more susceptible individuals at site 1.

Individual trees were inoculated at more than one time to confirm the consistency of the estimates of lesion length. For trees inoculated at three different times, lesion lengths were significantly correlated between all combinations of time points, indicating that results from any single time point were predictive of a tree's susceptibility. However, for the trees that were inoculated only twice (time 2 and time 3), lesion lengths at the two different time points were not significantly correlated. This reflected the fact that some trees sustaining short lesions in the first test had longer lesions in the second test, such that the ranking differed between the two tests. Thus, some trees were revealed as susceptible in only one of the two tests. The reasons for this discrepancy were unknown, but it may reflect a differential effect of environmental factors (such as temperature) on tree responses to inoculation. In general, it seems reasonable to conclude that, whereas long lesions may be taken as conclusive evidence of susceptibility, more limited lesion development may reflect the operation of limiting factors other than host resistance. Consequently, two independent tests should be considered the minimum necessary to confirm that a tree is resistant to pitch canker.

Whereas apparent differences in susceptibility between tests may reflect environmental influences, altered resistance of the host may also be a factor. For example, Monterey pines subjected to multiple inoculations with the pitch canker pathogen showed a progressive decrease in lesion length over time (Schmale *et al*., 1999; Bonello *et al*., 2001). The findings presented here document a similar trend in bishop pine, in which the 50 trees that were inoculated three times exhibited a 63% decrease in mean lesion length from time 1 to time 2. These results indicate a response consistent with systemic induced resistance (SIR). Moreover, this effect cannot be attributed to the wounding associated with prior branch removal, which was shown to have no significant effect on lesion length.

To better resolve the differences among trees, the incubation time following inoculations at time 3 was lengthened from 10 to 13 weeks, to allow more time for lesion development. This may help to explain the 45% increase in mean lesion length, relative to the time 2 inoculations. Thus, to the extent that induced resistance was operative, it appears to have been negated by the longer incubation period. However, time 3 lesion lengths were still 33% shorter than those observed following time 1 inoculations. Experiments using clonal material and controlled environmental conditions are needed to confirm that SIR is operative in bishop pine.

Trees sustaining short lesions (approximately 5 mm) at all three inoculation times (four out of 50 trees tested) may prove to be useful sources of material for future studies on the genetic and biochemical bases of resistance to pitch canker, and ultimately for management of the disease. Where regeneration occurs in native forests, natural selection may enrich the population for individuals with resistance to pitch canker. Under other circumstances it may prove advantageous to utilize vegetative cuttings and/or seed from resistant individuals to minimize damage caused by pitch canker.

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