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Susceptibility of Five Landscape Pines to Pitch Canker Disease, Caused by *Fusarium subglutinans* f. sp. *pini*

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Abstract. **Pitch canker, caused by *Fusarium subglutinans* f. sp. *pini*, causes branch dieback and stem cankers in many species of pine. Monterey pine (*Pinus radiata* D. Don), one of the most widely planted pines in the world, is extremely susceptible to pitch canker. Four other pine species, which might serve as alternatives to Monterey pine in landscape settings, were found to be relatively resistant, based on the size of lesions resulting from branch inoculations under greenhouse conditions. Of these species, Japanese black pine (*P. thunbergiana* Franco) was the most resistant, followed by Canary Island pine (*P. canariensis* Sweet ex K. Spreng), Italian stone pine (*P. pinea* L.), and Aleppo pine (*P. halepensis* Mill.). Consistent with these findings, a field survey conducted in Alameda County, Calif., revealed Monterey pine to have the highest incidence of infection, with significantly lower levels in Aleppo, Canary Island, and Italian stone pines. Japanese black pine was not observed in the survey area.**

Pitch canker, caused by *Fusarium subglutinans* f. sp. *pini*, was first described in 1946 (Hepting and Roth, 1946) in the southeastern United States, where it remains a chronic problem in plantations, seed orchards, and nurseries (Dwinell et al., 1985). The disease was discovered in California in 1986 (McCain et al., 1987) and has since been reported in Mexico (Rodriquez, 1989), Japan (Kobayashi and Muramoto, 1989), and South Africa (Viljoen et al., 1994). In California, pitch canker has spread rapidly through urban forests of Monterey pine and now threatens native populations of this species as well (Storer et al., 1997).

The initial symptom of pitch canker, fading of needles near the tips of young branches, is the result of branch and/or cone infections that develop into girdling lesions. Repeated infections, which eventually include older branches and the main stem, render trees unattractive and prone to premature death. In urban forests this necessitates a costly process of removal and replacement.

In California, most infections appear to result from feeding by various insect associates of conifers that carry the pitch canker pathogen. Principal among these insect vectors are engraver beetles, *Ips* spp. (Fox et al., 1991), twig beetles, *Pityophthorus* spp. (Fox et al., 1990), the cone beetle, *Conophthorus radiatae* Hopkins, and the dry twig and cone beetle, *Ernobius punctulatus* Fall (Hoover et al., 1995, 1996).

In the United States, the pitch canker pathogen infects 23 pine species and Douglas-fir (Storer et al., 1997). In California, although Monterey pine is the species most widely affected by the disease, 11 other conifer species have sustained natural infections (Storer et al., 1997). Based on the incidence of diseased trees, Monterey pine appears to be more susceptible than other pines grown as landscape trees in California. However, no quantitative data on the relative susceptibility of these species are available. Thus it is unclear to what extent a lower incidence of infection reflects inherent differences in host susceptibility to pitch canker, as opposed to chance differences in proximity to inoculum sources and/or differences in the activity of insect vectors.

Because of the importance of Monterey pine as a landscape tree in Mediterranean climates throughout the world, identification of alternative species that will not sustain serious damage from pitch canker would be useful. Other pines are suitable for landscape plantings under climatic conditions where Monterey pine is now commonly grown. Any of these species with demonstrated resistance to pitch canker could be substituted for Monterey pines in pitch canker–infested areas.

This study was undertaken to evaluate the relative susceptibility to pitch canker of five species: Monterey pine, Aleppo pine, Canary Island pine, Italian stone pine, and Japanese black pine. For all five species, quantitative differences in response to mechanical inoculations were characterized, and, for all but Japanese black pine (which did not occur in the survey area), we recorded the incidence of disease on these species under field conditions.

Materials and Methods

Greenhouse experiments. Trees between 3 and 4 years of age, in 19-L pots, were obtained from Boething Treeland Farms (Portola Valley, Calif.) in Oct. 1995, after which they were maintained outdoors in Berkeley, Calif., under drip irrigation. All trees were healthy in appearance with no evidence of disease or insect problems. Two weeks before an experiment, the trees to be inoculated were moved into a greenhouse. Three inoculation experiments were conducted. During the first and third experiments, initiated in Feb. 1996 and June 1996, respectively, trees were maintained in a greenhouse with supplemental (nighttime) heating where temperatures ranged from 24 to 35 °C and 23 to 45 °C, respectively. In the second experiment, initiated in Mar. 1996, temperatures were not regulated and ranged from 10 to 36 °C.

The isolate used to inoculate trees, FSP 34, was selected because it is representative of the predominant strain (VCG C1) found in California (Correll et al., 1992; Gordon et al., 1996). Inoculum was obtained by growing FSP 34 on potato dextrose agar at room temperature, 23 ± 2.5 °C, for 10–14 d. Spore suspensions were prepared by flooding plates with sterile water and gently scraping them with a sterile glass slide. The suspended fungal biomass was filtered through a double layer of sterile cheesecloth that retained mycelium and allowed most of the spores to pass through. The concentration of spores in the suspension, estimated using a hemacytometer, was adjusted by the addition of sterile water to either 5000, 50,000, or 500,000 spores/mL. Concentrations were confirmed by spreading 0.1 mL of a dilution of each spore suspension (1:10, 1:100, and 1:1000 for the low, medium, and high concentrations, respectively) over the surface of potato dextrose agar in petri dishes and counting the number of germinating spores 24–48 h later.

Inoculation courts were prepared on second-year wood of young branches by hand-drilling a 1.6-mm-diameter hole through the bark to the sapwood. A 5-µL drop of spore suspension was placed in the wound site. Each tree was inoculated once with each of the three different spore suspensions, such that the number of spores delivered was either 25, 250, or 2500. Control inoculations were performed by depositing sterile water in the wound site instead of a spore suspension. Each inoculation was placed on a separate branch.

Each of the three inoculation experiments included five trees of each of five pine species. All inoculated branches were cut from the tree 35 d (Expt. 1) or 41 d (Expt. 2) after they were inoculated. Bark was removed from the area

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surrounding the inoculation site and the length of the lesion, as evidenced by discoloration, was measured to the nearest mm.

Data from the first two inoculation experiments were analyzed using analysis of variance (ANOVA) and the significance of differences among treatment means was assessed using Tukey's all pairwise comparisons test. In the third experiment, inoculated branches were allowed to remain on the trees for 135 d, when a final rating was taken for chlorosis and/or death of needles distal to the point of inoculation.

Survey of urban trees. The incidence and severity of pitch canker in pines planted along roadways in Fremont and Union City, Alameda Co., Calif. were assessed in Jan. 1997. Four routes were selected along which all pines were rated for symptoms of pitch canker. These routes were chosen in areas where pitch canker was known to occur and where the trees were planted in proximity to public roads, facilitating evaluation of their disease status.

Pitch canker infections were identified visually. Individual branch infections were recognized as tips with reflexed discolored needles or tips from which needles had fallen. Cankers on the main stem were identified by their copious production of resin, which flowed down the stem. The association of these symptoms with pitch canker disease was established previously, through isolation of the pathogen from the symptomatic tissue (Correll et al., 1991, 1992; Gordon et al., 1996). The severity of pitch canker was characterized by placing trees into one of three categories based on the number of branch tips with symptoms of pitch canker: 1) no infections, 2) 1–10 symptomatic tips, or 3) >10 symptomatic tips. For trees with one or more symptomatic tips, the presence or absence of recent infections (needles retained on the branch) and old infections (branch tip with no needles) was noted. The number of cankers on the main stem and larger branches of the tree was classified as 0, 1–3, or >3 per tree. A total of 736 trees was included in the survey. Hierarchical log-linear analyses were used to determine the relationships between pitch canker severity and pine species.

Results

Greenhouse inoculations. Data from the first two inoculation experiments were log transformed to eliminate a relationship between the mean and variance evident in the nontransformed data, and analyzed using a three-way ANOVA. No lesions were observed on branches inoculated with water alone and these were excluded from the analysis.

All three factors, pine species, spore load, and date of inoculation (i.e., experiments conducted in Feb. or Mar. 1996), had significant effects on lesion length ($P < 0.001$). There was no significant interaction between species and spore load ($P = 0.319$) or between species and date of inoculation ($P = 0.885$). However, the interaction between spore load and date was significant ($P = 0.019$). This interaction and the significant effect of inoculation date can both be attributed to a difference in the response of trees to inoculation with the highest dose, 2500 spores, in the first and second experiments (Fig. 1).

Because of the spore load × date interaction, lesion lengths were compared between species only within experiments. The lesion lengths averaged over spore loads within each experiment (Fig. 2) show the ranking of the five species to be the same for both experiments. Mean lesion length on Monterey pine was significantly greater than that on each of the other four species. Similarly, the mean lesion length was significantly greater on Aleppo than on Japanese black pine; none of the other pairwise comparisons revealed any significant differences.

In the third experiment, foliar symptoms were evaluated 60 d after inoculation. At that time, 60% (3/5) of the Monterey pine branches inoculated with 2500 spores bore yellowing needles at and distal to the inoculation site. At 90 d after inoculation, the remaining two Monterey pine branches inoculated with 2500 spores were symptomatic, as were 60% (3/5) of the branches inoculated with 250 spores and 20% (1/5) of those inoculated with 25 spores. At 135 d after inoculation, no additional Monterey pine branches and none of the inoculated branches on any of the other four pine species showed any foliar symptoms.

Survey of urban trees. Monterey pine, Aleppo pine, Canary Island pine, and Italian stone pine were all encountered during the survey. Branches showing symptoms of pitch canker were observed only on Monterey, Canary Island, and Aleppo pines (Table 1). Main stem cankers were observed only on Monterey pine (Table 1). The percentage of trees with at least one symptomatic branch differed significantly at $P \leq 0.05$ for all pairwise comparisons of species, with Monterey pine being most susceptible and Italian stone pine the least susceptible.

In Monterey pine, the percentage of branches showing recent symptoms of pitch canker was greater on trees with old branch tip infections than on trees with no old infections (52.3% vs. 9.1%, $G = 36.89$, 1 df, $P < 0.001$). The occurrence of main stem cankers was

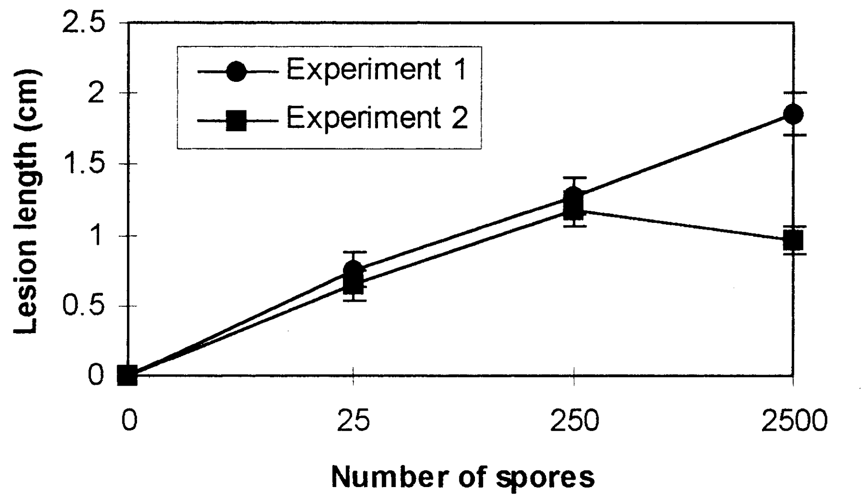

Fig. 1. The relationship between lesion length, averaged over five pine species, and the number of spores of *Fusarium subglutinans* f. sp. *pini* used to inoculate each branch. Each data point corresponds to the lesion length at the site of inoculation; error bars represent the standard error of the mean. Experiments 1 and 2 correspond to the first and second greenhouse inoculation experiments, which were initiated in February and March, respectively.

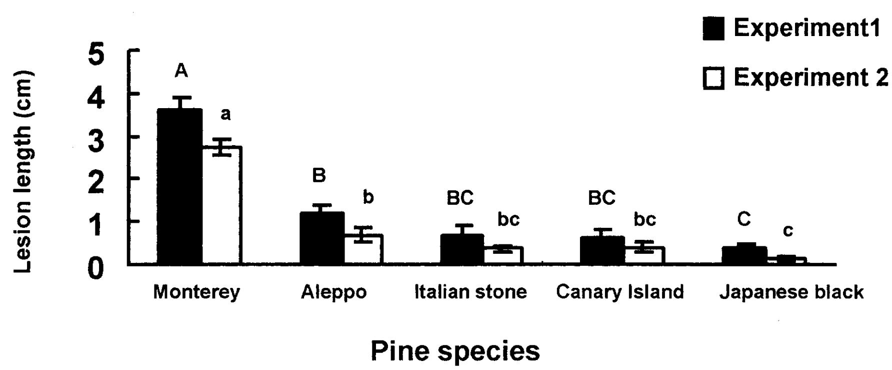

Fig. 2. Mean lesion size (averaged over inoculations with three different spore loads: 25, 250, and 2500) for each of five pine species inoculated with *Fusarium subglutinans* f. sp. *pini* on two different dates. Experiments 1 and 2 correspond to the first and second greenhouse inoculation experiments, which were initiated in February and March, respectively. Mean lesion lengths denoted by a common letter are not significantly different at $P = 0.05$ (uppercase letters for Experiment 1 and lower case letters for Experiment 2; comparisons are within experiments only).

Table 1. Severity of pitch canker disease on four species of pine.[z]

	Percentage of trees in each severity class based on number of:					
	Infected branches[y]			Infections on main stem[x]		
Host species	0	1–10	>10	0	1–3	>3
Monterey pine (n = 170)	35	36	29	68	16	16
Aleppo pine (n = 94)	91	9	0	100	0	0
Canary Island pine (n = 320)	97	3	0	100	0	0
Italian stone pine (n = 152)	100	0	0	100	0	0

[z]The pines evaluated were street trees encountered along four survey routes in Fremont and Union City (Alameda Co., Calif.).
[y]Severity classes are based on the numbers of infected branch tips: 0, 1–10, or >10.
[x]Severity classes are based on the numbers of cankers on the main stem of the tree: 0, 1–3, or >3.

greater on trees showing tip mortality than on trees without tip mortality (48.6% vs. 1.7%, $G = 49.50$, 1 df, $P < 0.001$), and on trees with old branch infections than on those without old infections (51.4% vs. 1.5%, $G = 57.57$, 1 df, $P < 0.001$). Lastly, main stem cankers occurred on a greater percentage of trees that had more than ten branch tip infections than on trees with between one and ten infections (66.0% vs. 34.4%, $G = 11.00$, 1 df, $P < 0.01$).

Discussion

Since pitch canker was first observed in California in 1986, thousands of infected trees have been killed by the disease and associated bark beetle attacks; thousands more have sustained so much damage as to have entirely lost their aesthetic value. In many areas where diseased Monterey pines are found, exotic pine species (i.e., landscape pines not native to California) occur in close proximity. Of these species, Aleppo pine appeared to be the most frequently infected, followed by Canary Island and Italian stone pines (T.R. Gordon, D. Okamoto, A.J. Storer, and D.L. Wood, unpublished data). This same order of infection frequency was observed in the survey reported here.

The abundance of infected Monterey pines in the survey area constitutes a large reservoir of inoculum to which the exotic species are exposed. Thus the rarity of disease amongst the exotic species is not due to an absence of local inoculum sources. The low incidence of pitch canker infections on such exotic species may be due, in part, to a lack of visitation by insects carrying the pitch canker pathogen. Most infections on Monterey pine appear to result from the activity of native insects (Fox et al., 1991; Hoover et al., 1996), which only occasionally colonize exotic pines in central coastal California (D.L. Wood and P. Svihra, personal observations). Because we have no data on the frequency with which insects capable of vectoring pitch canker visit the exotic pines included in this study, we cannot estimate the extent to which this factor contributed to the low infection rates that were observed in the survey area.

Whereas insect feeding preferences may limit the frequency with which the exotic pines are challenged by the pathogen under field conditions, controlled inoculations document their inherent resistance to pitch canker, relative to Monterey pine. Furthermore, the ranking of species, based on lesion sizes resulting from mechanical inoculations, is very similar to the ranking based on infection frequency in the field. Aleppo pine, which had the highest infection frequency of the exotic pines in the field, also had the largest mean lesion size, after Monterey pine, in controlled inoculations (Fig. 2). Thus, Aleppo pine appears to be significantly less susceptible to pitch canker than Monterey pine based on both lesion size and the incidence of disease recorded in the survey. Moreover, no foliar symptoms developed on Aleppo pine in the greenhouse, even on branches inoculated with 2500 spores (data not shown); this is far more than an insect is likely to deliver to an infection court in the field (T.R. Gordon, D. Okamoto, A.J. Storer, and D.L. Wood, unpublished data). Thus, other predisposing factors may be required to establish visible infections under field conditions.

Japanese black pine had the smallest mean lesion size of the pines tested, 0.39 ± 0.09 cm and 0.15 ± 0.05 cm, in the first and second experiments, respectively. Thus, in Japanese black pine, mean lesion sizes were only slightly larger than the wound created for the inoculation (0.16 cm). Muramoto et al. (1993) reported more extensive lesion development in their inoculations of Japanese black pine, though branch dieback was not observed. The larger lesions may reflect a more aggressive method of inoculation; an area of 4 cm^2 was inoculated by Muramoto et al., compared with only 0.02 cm^2 in our study. Differences in isolate virulence and/or susceptibility of the host genotype may also contribute to the difference in the extent of lesion development between the two studies.

Japanese black pine is not as widely planted in California as the other exotic species tested, and did not occur in the area surveyed for pitch canker, precluding confirmation of resistance based on disease incidence under natural conditions. However, no infections have been observed on this species in California (T.R. Gordon, D. Okamoto, A.J. Storer, and D.L. Wood, unpublished data), consistent with the conclusion, from greenhouse studies, that Japanese black pine is resistant to pitch canker under field conditions. The same appears to be true in Japan, where Muramoto, et al. (1993) reported the isolation of the pitch canker pathogen from bark on healthy Japanese black pines but did not observe any naturally occurring infections on this species.

Natural pitch canker infections have been reported for both Canary Island and Italian stone pine (Correll et al., 1991), although no infections were observed on Italian stone pine in the present study. Mean lesion sizes for these species (≈0.5 cm in both cases) were only slightly larger than for Japanese black pine. The small lesion sizes on Canary Island and Italian stone pine and the rarity with which natural infections are observed suggest that both species are unlikely to sustain serious damage from this disease. The occasional infected Canary Island and Italian stone pines may represent rare susceptible individuals within otherwise resistant species. However, even the trees that have pitch canker are apparently relatively resistant, as none of them was heavily infected. Of the infected Canary Island pines seen in the present study, most had only a single infected tip, none had more than three, and none had stem cankers.

The results of this study indicate that several species well adapted to Mediterranean climates are either moderately or highly resistant to pitch canker. The correspondence between the extent of lesion development in mechanical inoculations and frequency of infection in the field suggest that greenhouse screening tests are predictive of resistance under natural conditions. Of the species tested, Japanese black pine appears to be the most resistant, based on the extent of lesion development. However, both Canary Island and Italian stone pine were comparable with Japanese black pine in greenhouse tests, and natural infections are rare. Consequently, all of these three species would be expected to sustain little or no damage from pitch canker and would therefore be appropriate for landscape plantings where this disease is prevalent. Of course, none of these species is necessarily free of other pest problems; this consideration, along with general horticultural requirements for a landscape tree, should be taken into account in selecting the appropriate species for a particular location.

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