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Susceptibility of Douglas fir (*Pseudotsuga menziesii*) to pitch canker, caused by *Gibberella circinata* (anamorph = *Fusarium circinatum*)

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For better characterization of the risk of pitch canker (caused by *Gibberella circinata*, anamorph = *Fusarium circinatum*) to Douglas fir (*Pseudotsuga menziesii*), Californian isolates, selected exotic isolates, and ascospore progeny of a cross between wild-type Californian isolates were tested for aggressiveness to this host species. In addition, seedlings from representative provenances of *P. menziesii* in California were tested for susceptibility to pitch canker. The results revealed only minor differences between isolates, but differences in susceptibility between trees were often significant. The majority of the tested trees were relatively resistant as indicated by the development of only very short lesions, but some were clearly susceptible.

Keywords: aggressiveness, disease resistance, forest pathology

Introduction

Pitch canker is caused by *Gibberella circinata* (synonym = *Gibberella fujikuroi* mating population H) [anamorph = *Fusarium circinatum* (synonym = *Fusarium subglutinans* f.sp. *pini*)]. The disease affects numerous pine species grown in plantations in the south-eastern USA (Dwinell *et al*., 1985) and has caused extensive damage to both native and planted *Pinus radiata* (Monterey pine) in California, USA (Gordon *et al*., 2001). Pitch canker has recently become a serious problem in South Africa (Viljoen & Wingfield, 1994), Spain (Landeras *et al*., 2005) and Chile (Wingfield *et al*., 2002), where exotic pines are grown for pulp and timber. Pitch canker has also been reported from Mexico (Guerra-Santos, 1999) and Japan (Muramoto & Dwinell, 1990). The only known host of the pitch canker fungus outside the genus *Pinus* is *Pseudotsuga menziesii* (Douglas fir), which was reported to be infected under field conditions in California (Gordon *et al*., 1996). The affected trees were planted (from an unknown seed source) and symptoms were restricted to branch dieback. Although the trees continued to decline and eventually died, it was unclear to what extent pitch canker contributed to their demise. The pitch canker pathogen has also been associated with tip dieback in native stands of *P. menziesii* (unpublished observations). Inoculations of trees in these stands indicated that *P. menziesii* was relatively resistant to pitch canker (Erbilgin *et al*., 2005).

In California, native *P. menziesii* is commonly found in close proximity to both planted and native stands of highly susceptible pine species such as *P. radiata* and *Pinus muricata* (bishop pine) (Gordon *et al*., 2001), which provides opportunities for susceptible individuals to become infected. Furthermore, *P. menziesii* has an extensive range in the western USA and has great importance as a commercial timber species. Consequently, it is of interest to know how much of a threat pitch canker represents to *P. menziesii*. To this end, the present study was undertaken to (i) determine if isolates of *G. circinata* differ in aggressiveness to *P. menziesii*; and (ii) test trees representative of populations found throughout the native range of *P. menziesii* in California for susceptibility to pitch canker.

Materials and methods

Trees

With the exception of the provenance study (described below), all experiments used 3- to 5-year-old trees (in 19-L pots) obtained from commercial nurseries in California. At least 2 weeks before inoculation trees were moved to either a glasshouse or growth chamber and maintained in the same location for the duration of the experiment. When trees were kept in a glasshouse, minimum and maximum daily temperatures are indicated where each experiment is

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described. For experiments conducted in a controlled-environment growth chamber, temperatures were maintained at 25°C during the 12-h light period and 18°C during the 12-h dark period. In all cases, each tree was used in an inoculation experiment only once.

To assess the susceptibility of *P. menziesii* within its native range, trees were grown from seed representative of populations in various provenances in California. Seed was obtained from collections established either by the California Department of Forestry and Fire Protection or the US Forest Service. These collections included two coastal and six inland provenances in California. Seeds were sown in 0·65-L tubes (D-40s, Steuwe and Sons) containing sphagnum peat : perlite (3 : 1, v/v) with Osmocote 18-6-12 controlled-release fertilizer (Scotts Co.) at 3·8 kg m^{-3} and Micromax Micronutrients (Scotts Co.) at 0·7 kg m^{-3}. Seedlings were grown in a glasshouse located at Pebble Beach, CA, USA. Water was applied as needed and at least once each week included 1·0 mL L^{-1} of a water-soluble fertilizer (Excel 21-5-20, Scotts Co.). When trees reached *c*. 1 year old they were transported to Davis, CA. At Davis, trees were maintained in a glasshouse for up to 2 months before being moved to a controlled-environment growth chamber, where they were allowed to equilibrate for a minimum of 2 weeks prior to inoculation. Conditions in the growth chamber were as described above.

Inoculation procedures

Inoculum was prepared by washing conidia from cultures growing on potato dextrose agar (PDA) using 0·5% KCl, as described by Schmale & Gordon (2003). A haemocytometer was used to quantify inoculum density, and spore viability was assayed by plating appropriate dilutions on PDA and counting colonies 48 h later. With the exception of seedlings used in the provenance study, trees were inoculated by making a wound with a 1·6-mm drill bit on a 2-year-old branch, and depositing 5 μL of a spore suspension therein as described by Gordon *et al*. (1998a). The estimated number of spores delivered is specified for each experiment. Inoculated branches were rated by measuring the length of the lesion at the inoculation site following the incubation period, which is specified for each experiment.

For provenance seedlings, a single inoculation was placed on the main stem at the junction of succulent and lignified tissue. In this case, inoculum was delivered in a volume of 2 μL and the target dose was 50 spores. This dose was adopted because higher inoculum levels, such as those applied to 2-year-old branches, appeared to result in overestimated susceptibility in other species (unpublished data). Inoculated trees were rated as described above.

Dose comparisons

Two *P. menziesii* trees were inoculated with a known aggressive isolate of *G. circinata* (GL 289), which is associated with the predominant vegetative compatibility group (VCG) in California (C1) (Gordon *et al*., 1996). Three spore loads were used: target doses were 50, 125 and 250 spores. On each tree, three separate branches were inoculated with each spore load, for a total of nine inoculated branches per tree. One additional branch on each tree was wounded in the same manner as inoculated branches, but water was deposited into it instead of inoculum (mock inoculation). Two trees of a known susceptible species (*P. radiata*) of approximately the same age were included for comparison, and were inoculated in the same manner. The first replication was conducted in a lathhouse, where mean daily maximum and minimum temperatures were 29·1 and 15·2°C, respectively. For the second and third replications, trees were maintained in a glasshouse where mean maximum and minimum temperatures were 24·0 and 15·6°C, respectively, for the second replication, and 24·2 and 18·1°C, respectively, for the third. Incubation periods for these replications were 33, 38 and 42 days, respectively.

Table 1 Isolates of *Gibberella circinata* representative of different geographical regions tested for aggressiveness on *Pseudotsuga menziesii*

Isolate	Other designation[a]	Origin[b]	Lesion length (mm)[c] Rep 1	Rep 2
GL 293	NA	NC, USA	3·1 ± 0·8	4·4 ± 2·4
GL 294	NA	GA, USA	2·4 ± 0·5	3·9 ± 2·1
GL 295	LJ	Mexico	2·8 ± 0·3	4·5 ± 2·3
GL 299	T5	Mexico	4·3 ± 3·2	3·9 ± 0·8
GL 296	SA0009	South Africa	3·4 ± 0·5	5·9 ± 5·8
GL 297	SA0024	South Africa	2·6 ± 1·3	3·5 ± 0·4
GL 298	SA-GE 37	Japan	2·3 ± 0·3	5·0 ± 2·2
GL 291	NA	CA, USA	2·3 ± 0·5	2·8 ± 0·3

[a]NA = no previous published reference to this isolate. All others, Wikler & Gordon (2000).
[b]Location from which the isolate was originally obtained.
[c]Mean lesion length ± SD, based on four trees for each replication.

Exotic isolates

Four *P. menziesii* trees were inoculated with eight *G. circinata* isolates of diverse geographical origin (Table 1). Each isolate was inoculated into a single branch on each of the four trees (target dose, 250 spores per inoculation). A mock inoculation was applied to a single branch on each tree. Two trees of a known susceptible species (*P. muricata*), similar in age to *P. menziesii*, were inoculated in the same manner. Trees were maintained in a growth chamber under conditions described above. Incubation periods were 31 and 28 days for the two replications of the experiment.

Resident Californian isolates

Six isolates of *G. circinata* (representing VCGs C1, C5, C7, C8, C9 and C10), originally obtained from pitch canker-affected *P. radiata* in California, were tested for their pathogenicity on *P. menziesii*. Each isolate was

inoculated into three separate branches, for a total of 18 inoculated branches per tree, on each of four *P. menziesii* trees (target dose, 250 spores per inoculation). For comparison, two *P. radiata* trees of approximately the same age were inoculated in the same manner on three branches per tree for each of the six isolates. A mock inoculation was applied to a single branch on each tree. Trees were maintained in a growth chamber under conditions described above. Incubation times for the two replications of this experiment were 32 and 31 days, respectively. A similar experiment was conducted with Californian isolates representing VCGs C1, C3 and C6, each of which was inoculated into three branches on each of four trees. Incubation times for the two replications of this experiment were 32 and 31 days.

Ascospore progeny

Ascospore progeny to be tested for pathogenicity were obtained by crossing two *G. circinata* isolates as described by Wikler *et al.* (2000). Individual ascospores were recovered and used to establish cultures, which were stored on dried filter paper at 4°C. Each of the progeny used in inoculations was determined to be associated with a different VCG of *G. circinata* and thus represented a unique product of sexual recombination (unpublished data).

Twenty-four ascospore progeny were tested for pathogenicity on *P. menziesii*. In the first set of inoculations, 12 progeny and both parental isolates were each inoculated twice on a single branch on each of four trees, for a total of 28 inoculations on 14 branches on each tree. Distal and proximal inoculation sites on the same branch were separated by *c.* 10 cm. Trees were maintained in a glasshouse, where mean maximum and minimum daily temperatures during the 47-day incubation period were 30·2 and 17·4°C, respectively. The second set of 12 progeny (and both parental isolates) was tested in the same manner as the first. In this case, the incubation period was 46 days and mean maximum and minimum daily temperatures were 25·5 and 16·8°C, respectively.

All progeny were tested a second time with the same experimental design, except that progeny were divided into three groups instead of two; within each group, each isolate was inoculated twice on a single branch on each of four trees. These tests were conducted in a growth chamber with conditions as described above. Incubation periods for the three sets of trees were 29, 31 and 23 days. Four additional trees were used to evaluate six of the most aggressive progeny (based on the first two experiments). Each isolate was inoculated once on each of two branches on each of four trees. Trees were maintained in a growth chamber under the conditions described above and branches were rated 32 days after inoculation.

Provenance study

A total of 188 seedlings representative of eight California provenances of *P. menziesii* var. *menziesii* were inoculated with *c.* 50 spores of GL 291, using the procedure described above. Inoculated seedlings were rated 21 days after inoculation. A subset of inoculated seedlings (those with the longest lesions) was maintained for an additional 2 months to determine if lesions would lead to girdling of the inoculated stem.

Data analysis

ANOVA was used to assess the significance of differences in aggressiveness between isolates. Lesion-length data were fitted to a mixed linear model using the PROC MIXED procedure in SAS (SAS SYSTEM ver. 8·0; SAS Institute), with 'isolate' treated as a fixed effect and 'replication', 'tree' and interaction terms treated as random effects. Degrees of freedom were calculated using the Satterthwaite method (Gaylor & Hopper, 1969). Levene's test was used to confirm homogeneity of variances. Pearson's product-moment correlation coefficient was calculated using SPSS ver. 12·0 (SPSS Inc.)

Results

Dose comparisons

The estimated mean numbers of viable conidia delivered (±SD) for target doses of 50, 150 and 250 spores were, respectively, 100 ± 25, 160 ± 0 and 245 ± 15 for the first replication of the experiment; 39 ± 3, 125 ± 8 and 332·5 ± 28 for the second replication; and 49·5 ± 4, 90 ± 5 and 218 ± 28 for the third. For the first experiment, lesion lengths on *P. menziesii* branches ranged from 2·5 to 8·0 mm at both 50 and 125 spores per inoculation, and from 3·5 to 13·5 mm at 250 spores per inoculation (N = six branches for each target dose). Similar results were obtained in two subsequent replications of the experiment and, based on all three replications (N = 54), there was no significant correlation between dose (as estimated by spore viability) and lesion length (r = –0·08; P = 0·394). Comparable inoculations of *P. radiata* branches induced mean lesion lengths of 21·0, 21·1 and 22·5 mm at target doses of 50, 125 and 250 spores, respectively. The length of the discoloured region on water controls was similar for both species, and was equal to, or only slightly greater than, the size of the wound (1·6 mm).

Exotic isolates

In the first experiment, mean lesion lengths induced by eight isolates (averaged over four *P. menziesii* trees) ranged from 2·3 to 4·3 mm. In contrast, lesion lengths for the same set of isolates on *P. muricata* ranged from 14·0 to 59·5 mm. The discoloured area on water-inoculated branches approximated the size of the wound. The longest lesion on *P. menziesii* was 9·0 mm and was induced by isolate GL 293 (from North Carolina, USA); otherwise the longest lesion on *P. menziesii* was 4·5 mm. Spore viability was lower than the target level of 250 spores per dose for most isolates, but even the lowest dose, 137·5 ± 8 spores for GL 291, was sufficient to induce lesions of 36·0–54·5 mm on *P. muricata*, and there was no significant correlation

between number of viable spores and lesion length on this susceptible species (r = 0·099; P = 0·408).

Similar results were obtained when four additional trees of *P. menziesii* were inoculated, except that the longest lesion in this case was 14·5 mm (induced by GL 294). In this experiment, inoculum for all isolates was within the range of the target dose ±13%. Based on analysis of the combined data set, the effects of replication and isolate were not significant (P = 0·99 and P = 0·198, respectively). Mean lesion lengths induced by each isolate on *P. menziesii*, averaged across four trees, are presented separately for the two replications of the experiment (Table 1). Differences between trees were not large, with mean lesion lengths, averaged across all isolates, ranging from 3·1 to 6·7 mm. However, as a factor in the analysis, tree was a significant source of variation (P < 0·01).

Resident Californian isolates

In the first experiment, isolates representing six VCGs were tested on four *P. menziesii* trees. Lesion lengths ranged from 3·5 to 45·0 mm, with an overall mean of 15·2 mm (N = 72). For comparison, lesion lengths induced by the same isolates on *P. radiata* ranged from 35·9 to 49·0 mm, with an overall mean of 43·9 mm (N = 23). Averaged across all six isolates, the mean dose delivered was 293 ± 39 spores. Lesion length was not correlated with the number of spores delivered (r = 0·194, P = 0·102). Similar results were obtained when this experiment was repeated, with an estimated mean of 288 ± 49 viable spores per inoculation.

Analysis of the combined data set showed no significant effect of replication (P = 0·403) or isolate × replication interaction (P = 0·89). The effect of tree was significant (P < 0·01), reflecting the fact that mean lesion lengths on eight trees (averaged across all isolates) ranged from 3·1 ± 1·0 to 17·6 ± 9·6 mm. On the other hand, the effect of isolate was not significant (P = 0·325). Mean lesion lengths induced by each isolate on *P. menziesii*, averaged across eight trees, are shown in Table 2.

The same experimental design was used to test two additional Californian isolates, corresponding to VCGs C3 and C6, along with VCG C1 (which provided a point of comparison with the experiment described above). The effects of replication and replication × isolate interaction were again not significant (P = 0·527 and P = 0·157, respectively). The effect of tree was significant (P < 0·01), with mean lesion lengths, averaged across three isolates, ranging from 9·1 ± 10·1 to 55·3 ± 17·2 mm. Although the effect of isolate was not significant (P = 0·199), there was a clear trend toward greater aggressiveness in the C3 isolate, which induced conspicuously longer lesions relative to the other two isolates on four of the eight trees tested. Averaged over all eight trees, mean lesion lengths were 20·7 ± 15·6, 33·9 ± 20·7 and 18·4 ± 15·0 mm for isolates associated with VCGs C1, C3 and C6, respectively.

Table 2 Californian isolates of *Gibberella circinata* representing six vegetative compatibility groups (VCG) tested for aggressiveness on *Pseudotsuga menziesii*

Isolate	VCG	Lesion length (mm)[a]
GL 289	C1	12·5 ± 7·4
GL 311	C5	14·9 ± 11·0
GL 312	C7	12·8 ± 7·3
GL 313	C8	13·4 ± 9·2
GL 314	C9	10·9 ± 8·7
GL 315	C10	11·1 ± 5·5

[a]Mean lesion length ± SD (N = 8).

Ascospore progeny

The first experiment included 12 progeny and both parental isolates, each being inoculated into two locations (distal or proximal) on the same branch (see Materials and methods) on each of four trees. In a preliminary analysis, location nested within tree was included as a random factor and was found not to have a significant effect on lesion length (P = 0·480). Thereafter, proximal and distal lesion lengths were averaged for each of the progeny on each tree tested.

Further analysis indicated that the effect of tree was significant (P < 0·01), but the effect of isolate was not (P = 0·100). The significant effect of tree was attributable to the much greater susceptibility of one tree, which sustained a mean lesion length (averaged across all isolates) of 31·3 ± 3·1 mm, relative to the other three trees, which had mean lesion lengths of 4·4 ± 0·2, 4·5 ± 0·2 and 7·8 ± 0·9 mm. Mean lesion lengths recorded for isolates on three trees sustaining short lesions (resistant trees) are presented separately from those measured on the more susceptible tree (Table 3). Although the isolates tested induced a much

Table 3 Inoculum doses and lesion lengths induced in *Pseudotsuga menziesii* by two interfertile isolates of *Gibberella circinata* and 12 of their ascospore progeny

		Lesion length (mm)[c]	
Isolate[a]	Spore viability[b]	Susceptible tree	Resistant trees
P1	230 ± 21	17·3 ± 13·1	5·3 ± 2·7
P2	235 ± 7	38·8 ± 5·3	5·0 ± 1·2
P3	257 ± 32	32·3 ± 8·8	6·0 ± 2·7
P4	250 ± 21	57·3 ± 17·3	9·2 ± 7·1
P6	277 ± 11	43·0 ± 2·8	4·8 ± 1·0
P8	182 ± 25	34·5 ± 3·5	4·8 ± 1·9
P9	247 ± 25	28·5 ± 1·4	4·8 ± 1·3
P11	207 ± 11	15·3 ± 1·1	4·2 ± 0·7
P13	257 ± 18	38·3 ± 4·6	8·8 ± 6·0
P17	242 ± 53	19·0 ± 8·5	5·2 ± 0·7
P19	222 ± 11	52·3 ± 6·0	6·4 ± 2·4
P23	305 ± 7	21·3 ± 6·7	4·3 ± 1·9
GL 290	285 ± 43	37·5 ± 13·4	5·8 ± 3·5
GL 301	250 ± 21	2·8 ± 1·8	3·3 ± 0·4

[a]GL 290 and GL 301 were the parent isolates.
[b]Estimated number of viable spores delivered in each inoculation.
[c]Mean lesion length ± SD (N = 2 and 6 for resistant and susceptible trees, respectively).

Table 4 Inoculum doses and lesion lengths induced in *Pseudotsuga menziesii* by two interfertile isolates of *Gibberella circinata* and 12 of their ascospore progeny

Isolate[a]	Spore viability[b]	Lesion length (mm)[c]
P10	222 ± 11	4·2 ± 1·5
P12	212 ± 18	3·5 ± 1·2
P25	235 ± 36	3·3 ± 1·2
P27	170 ± 57	3·8 ± 1·2
P28	175 ± 35	3·3 ± 1·2
P29	297 ± 53	3·5 ± 1·2
P30	297 ± 18	4·1 ± 1·2
P32	165 ± 14	3·6 ± 1·1
P36	217 ± 18	4·0 ± 1·4
P37	267 ± 11	4·0 ± 1·4
P38	215 ± 14	3·8 ± 1·2
P39	272 ± 11	4·0 ± 1·2
GL290	222 ± 18	3·7 ± 1·5
GL 301	195 ± 71	3·4 ± 1·3

[a]GL 290 and GL 301 were the parent isolates.
[b]Estimated number of viable spores delivered in each inoculation.
[c]Mean lesion length ± SD; values represent antilogs of statistics calculated from log-transformed data ($N = 8$).

Table 5 Lesion lengths induced by *Gibberella circinata* on Douglas fir from eight Californian provenances

Location[a]	Elevation (m a.s.l.)[b]	Mean lesion length (mm)[c]	Number tested
Humboldt: 40°36′ N 124°10′ W	152	2·4 ± 1·0	29
Mendocino: 39°29′ N 123°23′ W	762	3·0 ± 0·8	30
Placer: 39°08′ N 120°51′ W	1067	3·2 ± 2·4	22
Santa Cruz: 37°09′ N 122°16′ W	152	3·6 ± 1·9	32
Mariposa: 37°36′ N 119°54′ W	1067	4·6 ± 3·6	24
Tehama: 40°16′ N 121°46′ W	915	5·1 ± 3·7	25
Siskiyou: 41°19′ N 122°11′ W	1372	6·1 ± 5·0	12
Siskiyou: 41°39′ N 122°52′ W	1220	6·4 ± 5·9	14

[a]County in California and approximate latitude and longitude of collection site.
[b]Approximate altitude at which seeds were collected.
[c]Mean lesion length ± SD.

narrower range of lesion lengths on the more resistant trees (3·3–9·2 mm) than on the susceptible tree (2·8–57·3 mm), mean lesion lengths induced on resistant trees in response to each isolate were significantly correlated with lesion lengths induced by the corresponding isolates on the susceptible tree ($r = 0{\cdot}711$, $P = 0{\cdot}004$).

For most isolates tested, the viable inoculum delivered was close to the target dose of 250 spores (Table 3), and variation in viability does not appear to explain differences in aggressiveness among isolates. For example, the isolate with the lowest estimated spore viability (P8) produced a lesion length (34·5 mm) above the mean for all isolates on the susceptible tree (31·3 mm). Using all the data from the susceptible tree, there was not a significant correlation between spore load and lesion length ($r = 0{\cdot}033$, $P = 0{\cdot}455$).

Twelve additional progeny were tested and data were analysed as described for the first experiment. In this case, a log transformation was required to achieve homogeneity of variances. The effect of tree was significant ($P < 0{\cdot}01$), but the effect of isolate was not ($P = 0{\cdot}110$). Several of the progeny tested had spore viabilities well below the target dose of 250 (P27, P28 and P32 in Table 4), but lesion lengths induced by these isolates differed little from the overall mean of 3·8 mm.

When the same 24 progeny were tested a second time, they were divided among 12 trees instead of eight, reflecting the availability of fewer branches per tree. These tests for pathogenicity confirmed the results described above, in that no significant effect of isolate was detected (data not shown). The effect of tree was significant in one of the three experiments, in which mean lesion lengths (averaged over all isolates tested) ranged from 3·0 to 14·9 mm.

Six of the most aggressive progeny were tested on four additional *P. menziesii* trees and, once again, the effect of isolate was not significant (data not shown). However, tree was a significant factor ($P < 0{\cdot}01$). For three trees, mean lesion lengths, averaged across all six isolates, ranged from 5·2 ± 1·0 to 7·5 ± 1·2 mm, whereas the remaining tree sustained a mean lesion length of 31·3 ± 2·5 mm.

Provenance study

Mean lesion lengths on inoculated *P. menziesii* seedlings from eight Californian provenances ranged from 2·4 to 6·4 mm (Table 5). The longest lesion, 20·5 mm, was on a tree representing an inland provenance in Siskiyou County (Table 5). This lesion ultimately girdled the stem, something that was observed in only one other tree out of 58 retained for extended observation. The other tree with a girdling lesion was from an inland provenance in Mariposa County (Table 5).

Discussion

Dose comparisons indicated that the reaction of *P. menziesii* to the pitch canker pathogen was independent of the inoculum levels tested. Thereafter, a target dose of 250 spores was adopted as the standard for branch inoculations. Mock inoculations were included in the first six experiments (a total of 22 trees) and discoloration never extended much beyond the wounding site.

Isolates representing eight of 10 VCGs known to occur in California did not differ significantly in aggressiveness on *P. menziesii*. The two VCGs not tested (C2 and C4) are unlikely to differ from C1, to which they are clonally related (Wikler & Gordon, 2000). Previous work has shown isolates associated with C3 to be significantly more aggressive on *P. radiata* than isolates associated with other VCGs (Gordon *et al*., 1998b and unpublished data), and the data here indicate that the C3 isolate tested may also be more aggressive on *P. menziesii*. Although the observed differences were not statistically significant, this may have been the result of the limited susceptibility of most trees tested. In other words, the power to resolve inter-isolate

differences in aggressiveness is diminished where trees do not sustain large lesions. Documentation of greater aggressiveness in C3 isolates would support the view that this aspect of pathogenicity of *G. circinata* to *P. menziesii* is more-or-less proportional to that on pines. In this regard, it is noteworthy that isolates recovered from *P. menziesii* in nature do not appear to be fundamentally different from those found on the predominant host in California, *P. radiata*. All isolates obtained from *P. menziesii* have been associated with a VCG also found on pine hosts (Gordon *et al*., 1996), and have appeared similar in aggressiveness to isolates from *P. radiata*, suggesting no apparent specialization within *G. circinata* for pathogenicity on *P. menziesii*.

Isolates from four different parts of the world (southeastern USA, Mexico, Japan and South Africa) were not significantly more aggressive on *P. menziesii* than the Californian isolate with which they were compared. Thus there is no indication that limited variation in this component of pathogenicity to *P. menziesii* observed among resident Californian isolates is unusual for *G. circinata*. However, the present study used only a small sample of the worldwide population, and the possibility of undetected variation in pathogenicity to *P. menziesii* cannot be entirely dismissed.

The pitch canker fungus is known to reproduce both sexually and asexually (Britz *et al*., 1999), and wild-type Californian isolates can be crossed under laboratory conditions (Wikler *et al*., 2000). The available evidence suggests that clonal propagation predominates in California, at least in part because of limited overlap in distributions of the two mating types (Gordon *et al*., 1996). If sexual recombination does occur, new genotypes could manifest a greater degree of aggressiveness on *P. menziesii*. Sampling of recombinant progeny in the present study did not detect any such novel pathotypes, but more highly pathogenic forms could develop over time.

Whereas no experiment revealed a significant effect of isolate, tree was usually a significant factor. Thus variation in tree susceptibility appears more likely than isolate differences in aggressiveness to influence the impact of pitch canker on *P. menziesii*. However, even where the effect of tree was significant, actual differences in lesion length were often not large. The cut-off between resistance and susceptibility is somewhat arbitrary, but extensive testing of *P. radiata* and other pine species indicates that trees sustaining lesions consistently below 15 mm, under conditions used in the present study, are likely to manifest relatively little damage from pitch canker (Gordon *et al*., 1998a, 1998b; Storer *et al*., 1999; unpublished data). Applying this threshold to *P. menziesii*, of 54 trees obtained from retail nurseries, 11 would be placed in the susceptible category.

The provenance study identified only two clearly susceptible seedlings, and revealed no other indications of differential susceptibility among native populations. Collectively, these results suggest that *P. menziesii* is not at high risk of significant damage from pitch canker. However, it does seem likely that *P. menziesii* will commonly become infected where it is exposed to the pitch canker pathogen. Infections may be facilitated by movement of insect vectors to *P. menziesii* from more susceptible hosts such as *P. radiata* (Erbilgin *et al*., 2005). In most cases, lesion development would not be sufficient to cause girdling and dieback. Such cryptic infections could allow symptomless trees to serve as vehicles for movement of pitch canker to uninfested areas. In fact, transport of infected cuttings of *P. menziesii* appears to be the means by which the pitch canker pathogen arrived in New Zealand, where it was detected (and destroyed) by quarantine officials in 2003 (Vogler *et al*., 2004). This discovery highlights the need for better understanding of the relationship between *G. circinata* and other conifers currently regarded as nonhosts.

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