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Evidence for the occurrence of induced resistance to pitch canker, caused by *Gibberella circinata* (anamorph *Fusarium circinatum*), in populations of *Pinus radiata*

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Summary

Pitch canker, caused by *Gibberella circinata*, was discovered in California in 1986. Although initially quite damaging to Monterey pines (*Pinus radiata*), the severity of pitch canker has moderated in areas where the disease was first observed and some trees appear to have recovered completely. The absence of symptoms on trees that were once severely affected implies they have become more resistant to the disease. Experimental work has shown that *P. radiata* can manifest systemic induced resistance (SIR) in response to infection by the pitch canker pathogen and observations of disease remission may indicate that SIR is operative under natural conditions as well. As a test of this hypothesis, the susceptibility of trees in remission was assessed by inoculating them with *G. circinata* and recording the extent of lesion development. In addition, randomly selected trees in areas that differed in residence time of pitch canker were inoculated to determine if trees with a longer period of exposure to the pathogen were more resistant to the disease. The results of these tests showed that 89% of trees observed to be in remission sustained very limited lesion development, consistent with resistance to pitch canker. Furthermore, trees in areas where pitch canker was well established tended to be more resistant than trees in areas where the disease was of more recent occurrence. In sum, these findings support the view that SIR occurs in *P. radiata* and is contributing to a moderation of the impact of pitch canker under natural conditions.

1 Introduction

Long-lived tree species co-exist with parasitic microbes which, in the absence of pre-disposing stress, typically cause little or no damage to their host trees (Gordon et al. 2003). Presumably these meta-stable relationships reflect long periods of co-evolution, with selection militating against persistence of pathogens that cause severe disease on their preferred hosts (Burdon et al. 2006). The origins of most such relationships are lost to history but some likely were initiated when the vagaries of dispersal brought a microorganism into contact with a naïve host that could serve as a substrate for growth and reproduction. Recent examples of human-assisted movement of plant pathogens suggest that some such encounters may have devastating effects on populations of a new host (Anagnostakis 1987; van Mantgem 2004). Of course, less catastrophic outcomes must also have occurred and it seems likely that many encounters ended in failure for the parasite and hence left no evidence of the interaction.

Neither extreme success nor absolute, and hence cryptic, failure of an exotic pathogen can be expected to reveal mechanisms by which host populations adapt to introduced microbes. On the other hand, where susceptibility is intermediate, changes in host populations over time may offer insights into the means by which a destructive relationship transitions into one that allows for longer-term co-existence of host and parasite. Monterey pine (*Pinus radiata* D. Don) populations in California are of interest in this regard, in terms of their response to pitch canker, a disease caused by *Gibberella circinata* Nirenberg & O'Donnell.

Pitch canker was first recognized in California in 1986 (McCain et al. 1987), most likely following an introduction from the southeastern USA (Gordon 2006), where this disease was originally described by Hepting and Roth (1946). In California, *P. radiata* was initially severely affected by pitch canker (Storer et al. 2002; Wikler et al. 2003) but the impact moderated over time (Gordon 2006). As the range of the disease expanded, clear distinctions became apparent between stands in 'new areas', where pitch canker was of recent occurrence and disease severity increased rapidly, and stands in 'old areas', where the disease was of longer residence and the visual impact had diminished. To better understand the basis for this change, plots originally established in 1992 (Storer et al. 2002) were re-surveyed in 1999. This assessment revealed that many trees observed to be severely diseased in 1996 were disease-free 3 years later (Gordon et al. 2001). Previous work has shown that resistance to pitch canker can be elevated in trees as a result of prior exposure to the pathogen under controlled conditions (Bonello et al. 2001), and the aforementioned observations of disease remission suggest that this may occur under natural conditions as well (Gordon 2006).

The goal of the present study was to obtain experimental support for the proposition that enhanced resistance to pitch canker occurs naturally in *P. radiata* populations as a consequence of prior exposure to *G. circinata*. To this end, experiments were conducted for the purpose of testing two hypotheses concerning the impact of pitch canker on *P. radiata*: (i) trees in areas where pitch canker is well established tend to be less susceptible to the disease than trees only recently exposed to the pathogen and (ii) trees observed to be in remission are resistant to pitch canker.

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2 Materials and methods

2.1 Tests for an effect of time and location on susceptibility to pitch canker

To test for an effect of residence time of pitch canker on susceptibility of *P. radiata*, plots were established in areas where the disease was known to have been present for at least 10 years (old areas) and where the disease was of more recent occurrence (new areas). Based on the extent of disease development, residence time in new areas was estimated to be 2 years or less. To avoid introducing the pathogen to areas where it was not confirmed to occur, plots were located only in areas where the disease was already present (although all selected trees were disease-free in some cases). Within each area (old and new) eight plots were established in stands of *P. radiata*, where there was no evidence of tree removal (e.g. the presence of tree stumps), so comparisons would not be affected by the loss of highly susceptible individuals from old areas. All plots were comprised predominantly or exclusively of planted trees, with the exception of plots M1, M4 and M7 (Table 1) where stands appeared to have developed primarily through natural regeneration. In each plot, 20 trees were tagged and their diameters at breast height (DBH) were recorded. Each tree was also rated for severity of pitch canker, based on the number of infected branch tips and the number of cankers on the main stem, as described by Wikler et al. (2003). By this rating system, minimum tree disease severity was 0, and the maximum was 4.

Each tree was inoculated on each of three branches by creating a wound (approximately 1.6 mm in diameter) that penetrated the bark in the internodal area between terminal and penultimate branch whorls, and depositing therein a suspension of approximately 250 spores in 0.5% KCl, as described by Schmale and Gordon (2003). Spores were obtained from a known pathogenic isolate of *G. circinata* (GL 17) that was grown on potato dextrose agar and assayed for viability as described by Schmale and Gordon (2003). The spore germination assay confirmed inoculum viability to be within 92% of the target dose in all cases. Branches were harvested between 20 and 21 weeks after inoculation, and lesion lengths at sites of inoculation were measured to the nearest mm, as described by Gordon et al. (1998b). Where a lesion had girdled a branch, it was not always possible to accurately measure the lesion distal to the inoculation because the colour distinction between diseased and healthy tissue was not apparent. In these cases, lesion lengths were measured only proximal to the inoculation site and this value was doubled to provide an estimate of the full length of the lesion. All trees were inoculated first in 2002 and a second time, following the same procedure, 2 years later in 2004.

Table 1. Location and description of plots used to compare trees in areas differing in residence time of pitch canker.

			DBH[4]	Pitch canker		
Plot[1]	Location[2]	Status[3]	Mean ± SE	Incidence (%)[5]	Severity[6]	Inoculation date
M1	36°34′38″N, 121°54′41″W; 180 m	New	10.3 ± 1.3	0	0	08/05/2002
M2	36°35′24″N, 121°51′46″W; 42 m	New	17.8 ± 2.3	15	0.15	22/05/2002
M3	36°35′15″N, 121°49′42″W; 69 m	New	33.5 ± 2.6	60	0.80	22/05/2002
M4	36°36′00″N, 121°54′36″W; 102 m	New	21.4 ± 3.9	35	0.40	18/06/2002
M5	36°35′56″N, 121°54′41″W; 126 m	New	15.9 ± 3.5	25	0.25	18/06/2002
M6	36°38′43″N, 121°49′10″W; 41 m	New	21.9 ± 3.7	45	0.80	19/06/2002
M7	36°30′58″N,,121°56′17″W; 18 m	New	8.3 ± 0.8	0	0	10/07/2002
M8	36°35′00″N, 121°55′44″W; 200 m	New	10.7 ± 0.7	0	0	12/12/2002
SC1	36°57′00″N, 121°50′59″W; 102 m	Old	36.1 ± 1.8	35	0.40	07/05/2002
SC2	36°56′53″N, 121°50′51″W; 118 m	Old	38.0 ± 5.0	50	0.75	29/08/2002
SC3	36°55′50″N, 121°48′39″W; 74 m	Old	29.3 ± 4.0	65	0.80	05/06/2002
SC4	36°53′17″N, 121°49′46″W; 36 m	Old	51.3 ± 8.8	75	1.25	05/06/2002
SC5	36°55′02″N, 121°51′01″W; 24 m	Old	39.0 ± 2.8	60	0.80	05/06/2002
SC6	37°00′16″N, 121°58′40″W; 98 m	Old	56.4 ± 7.0	55	0.80	02/08/2002
SC7	36°59′40″N, 122°00′22″W; 94 m	Old	42.1 ± 7.5	35	0.35	02/08/2002
SC8	36°59′38″N, 121°59′59″W; 66 m	Old	38.7 ± 3.4	50	0.60	29/08/2002

[1]Plot numbers correspond to the following locations: M1 = Community Hospital of the Monterey Peninsula, M2 = Naval Post-Graduate School RV Park, M3 = City of Monterey Public Works Yard, M4 & M5 = City of Monterey Veteran's Memorial Park, M6 = State Highway One at Cal State Monterey Bay off-ramp, M7 = Pt. Lobos State Park, M8 = Pebble Beach Company Corporation Yard, SC1 & SC2 = State Highway One at Mar Monte off-ramp, SC3 = State Highway One at Buena Vista off-ramp, SC4 = Sunset State Beach, SC5 = Manresa State Beach, SC6 = Santa Cruz Gardens Park, SC7 & SC8 = DeLaveaga Park. Plots M1–M8 and SC1–SC8 are located in Monterey and Santa Cruz Counties, respectively.
[2]Approximate latitude, longitude and elevation of the plot center.
[3]Plots were categorized as 'Old' if pitch canker was known to be present in that area for 10 years or more, or 'New' if the disease was judged to have been present for $\leq$ 2 years.
[4]Mean diameter ± standard error (cm) of the main stem at breast height (n = 20).
[5]The percentage of trees with symptoms of pitch canker (n = 20).
[6]Mean disease severity of twenty trees in each plot on a scale of 0–4, with 0 corresponding to no disease and 4 to maximum disease severity, as described in section 2.1.

2.2 Tests for susceptibility of trees manifesting disease remission

In five plots that were established in 1992 (Storer et al. 2002), many trees were identified as severely diseased in 1996, based on the number of infected branch tips and/or the presence of cankers on the main stem of the tree. Some severely affected trees were observed to be disease-free in 1999 (Gordon et al. 2001) and a subset of these was selected for inoculation in 2004. Inoculations were accomplished using the procedure described above. After an incubation period of 20–21 weeks, branches were removed and rated for the extent of lesion development at the site of inoculation.

2.3 Data analysis

All analyses were conducted using sas (release 9.1; SAS Institute, Cary, NC, USA). Nonparametric tests were used because lesion length data were not normally distributed. Median lesion lengths were compared between new and old areas using the median test (Conover 1999). Differences in the distribution of lesion lengths between new and old areas were assessed using the Kolmogorov–Smirnov test (Sokal and Rohlf 1995). A chi-square test was used to determine if the proportion of resistant trees differed significantly between trees in remission and trees that were randomly selected with respect to their disease status.

3 Results

3.1 Tests for an effect of time and location on susceptibility to pitch canker

Disease incidence ranged from 0 to 60% in new area plots (disease present $\leq$ 2 years) and from 35 to 75% in old area plots (disease present $\geq$10 years) (Table 1), whereas disease severity (on a scale of 0–4) ranged from 0 to 0.8 and 0.35 to 1.25 in new and old area plots, respectively. Lesion lengths measured on three inoculated branches on twenty trees in each of 16 plots (eight each in new and old areas) were used to compute a mean for each tree. Considerable variation was observed within each plot but the overall mean lesion length was greater on trees in new areas (37.8 ± 2.8 mm) than in old areas (22.3 ± 1.8 mm) (Table 2). Relatively few lesions developed to the point that branches were girdled, but this occurred more frequently in new areas (11% of branches) than in old areas (3% of branches) (Table 2). Lesion length data were not normally distributed and hence nonparametric procedures were used to test for an effect of location (new *vs.* old areas) on lesion lengths. A median two

Table 2. Lesion lengths and branch mortality caused by *Gibberella circinata* on trees in areas differing in residence time of pitch canker.

		Lesion length[3]				
Plot[1]	Status[2]	Range[4]	Mean ± SE (mm)[5]	Median (mm)	<18 mm[6]	Branches killed[7]
M1	New	3.8–128	52.4 ± 9.4	55.0	0.35	0.21 (57)
M2	New	4.3–137	48.7 ± 10.2	31.0	0.40	0.25 (59)
M3	New	4.0–183	54.9 ± 11.5	39.3	0.35	0.26 (58)
M4	New	3.3–54.7	18.1 ± 3.5	11.7	0.65	0.02 (58)
M5	New	4.0–116	41.5 ± 7.6	35.6	0.35	0.10 (57)
M6	New	3.8–82.7	26.7 ± 4.9	28.3	0.40	0.02 (60)
M7	New	3.5–83.3	41.5 ± 5.7	42.8	0.20	0 (59)
M8	New	4.0–80.0	30.8 ± 5.7	23.7	0.40	0 (60)
SC1	Old	5.2–81.2	25.6 ± 4.3	24.4	0.35	0.02 (58)
SC2	Old	3.3–34.3	12.3 ± 2.0	9.0	0.75	0.02 (59)
SC3	Old	3.2–77.0	22.5 ± 4.4	14.6	0.55	0.03 (59)
SC4	Old	3.2–127	43.5 ± 7.7	31.0	0.20	0.03 (58)
SC5	Old	3.3–84.5	20.7 ± 4.7	11.4	0.60	0.02 (51)
SC6	Old	4.6–60.8	22.7 ± 4.2	14.6	0.65	0.07 (59)
SC7	Old	3.3–34.6	9.6 ± 1.8	6.3	0.85	0.00 (58)
SC8	Old	3.5–63.7	20.3 ± 4.0	13.8	0.65	0.05 (58)
SC1–8	Old	3.2–84.5	22.3 ± 1.8	13.6	0.58	0.03 (460)
M1–8	New	3.3–183	37.8 ± 2.8	27.4	0.38	0.11 (468)

[1]Plot numbers correspond to the following locations: M1 = Community Hospital of the Monterey Peninsula, M2 = Naval Post-Graduate School RV Park, M3 = City of Monterey Public Works Yard, M4 & M5 = City of Monterey Veteran's Memorial Park, M6 = State Highway One at Cal State Monterey Bay off-ramp, M7 = Pt. Lobos State Park, M8 = Pebble Beach Company Corporation Yard, SC1 & SC2 = State Highway One at Mar Monte off-ramp, SC3 = State Highway One at Buena Vista off-ramp, SC4 = Sunset State Beach, SC5 = Manresa State Beach, SC6 = Santa Cruz Gardens Park, SC7 & SC8 = DeLaveaga Park. Plots M1–M8 and SC1–SC8 are located in Monterey and Santa Cruz Counties, respectively. Aggregate values for all old and new area plots are represented by entries SC1–8 and M1–8, respectively.
[2]Plots were categorized as 'Old' if pitch canker was known to be present in that area for 10 years or more, or 'New' if the disease was judged to have been present for $\leq$ 2 years.
[3]Length of the lesion at the site of inoculation.
[4]The range of mean lesion lengths for each of 20 trees in each plot.
[5]Based on 20 trees in each plot.
[6]The proportion of trees with mean lesion lengths shorter than 18 mm (n = 20), an approximate threshold for resistance (Gordon et al. 1998b).
[7]The proportion of branches that were killed due to a girdling lesion; total number of branches evaluated in parentheses.

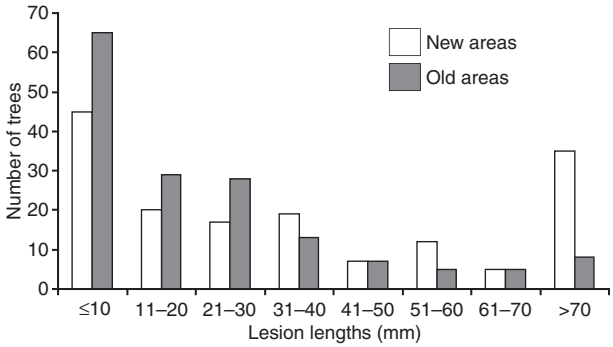

Fig. 1. The numbers of trees sustaining mean lesions lengths, induced by *Gibberella circinata*, within the ranges shown; based on 80 inoculated trees in each of two areas (new and old), which differed in residence time of pitch canker.

sample test (one-sided) showed median lesion length to be significantly greater (Z = 3.349, $p < 0.001$) in new (27.4 mm) than in old areas (13.6 mm). The two populations also differed in the distribution of lesion lengths, with a higher proportion of trees developing longer lesions in new than in old areas (Fig. 1). Based on a two-sample Kolmogorov–Smirnov (KS) test, the distributions of mean lesion lengths in new and old areas were significantly different (D = 0.269, $p < 0.001$).

An alternative to using three branch inoculations to calculate a mean for each tree is to treat data from each branch independently. Thus, $\sim$ 960 lesion lengths (one for each branch), instead of 320 tree means, can be used to compare susceptibilities to pitch canker in different areas. In this case, both median and KS tests showed the effect of location to be significant (Z = 5.013, $p < 0.001$ and D = 0.243558, $p < 0.001$, respectively). Another alternative is to regard only the longest lesion recorded for three inoculated branches as indicative of a tree's susceptibility. By this approach, median lesion lengths in new and old areas were 43.0 and 23.5 mm, respectively, and the effect of location was significant (Z = 3.9815, $p < 0.001$). Likewise, the distribution of lesion lengths differed significantly between the two areas (D = 0.294, $p < 0.001$).

Trees in new area plots were generally smaller (based on DBH) and thus presumably younger than trees in old areas (Table 1). Consequently, it is possible that longer lesions on trees in new areas were due, in part, to age (or size) related differences in susceptibility. To minimize the influence of these effects, a separate analysis was conducted using samples from both areas that were limited to trees with a DBH of <30 cm. With this restriction, mean lesion lengths were 37.7 ± 2.9 mm (n = 130) and 25.9 ± 3.4 mm (n = 58) for new and old areas, respectively. Median lesion lengths for new and old areas were 28.6 and 19.8 mm, respectively, and this difference was significant (Z = 2.835, p = 0.002). Lesion length distributions also differed significantly between the two areas (D = 0.270, p = 0.006). For trees with a DBH ≥30 cm, median lengths were significantly longer in new than in old areas (Z = 1.811, p = 0.035) but the distribution of lesion lengths was not significantly different (D = 0.265, p = 0.132).

All trees originally inoculated in 2002 were re-inoculated in 2004, with the exception of trees lost in the intervening period due to storm damage (12 in total). In 2004, mean lesion lengths were again longer in new (28.4 ± 2.0 mm, n = 152) than in old areas (22.7 ± 1.6 mm, n = 156). This difference was of lesser magnitude than in 2002 but the medians were still significantly different (Z = 2.2757, p = 0.011). The KS test showed differences in the distribution of lesion lengths between the two areas to border on significance (D = 0.153, p = 0.054).

Median lesion lengths for new area trees were 27.4 and 19.9 mm in 2002 and 2004, respectively, a difference that was significant according to a median test (Z = 2.262, p = 0.012). In addition, a KS test showed the distribution of lesion lengths to be significantly different between the 2 years (D = 0.181, p = 0.012). In contrast, median lesion lengths for old area trees were not significantly different in 2002 (13.6 mm) than in 2004 (15.8 mm) (Z = 0.903, p = 0.183) and likewise the distribution of lesion lengths did not differ significantly between years (D = 0.100, p = 0.402).

Table 3. Response of trees in remission to inoculation with *Gibberella circinata*.

Plot[1]	Location[2]	Mean ± SE[3] (n)	Range[4]	<18 mm[5]
New Brighton State Beach	36°58′49″N, 121°56′00″W; 40 m	10.4 ± 1.2 (20)	3.7–26.7	0.95 (19)
Soquel High School	36°59′34″N, 121°57′31″W; 30 m	6.8 ± 1.1 (12)	3.3–14.3	1.0 (12)
De Laveaga Golf Course	36°59′45″N, 122°00′16″W; 98 m	11.7 ± 2.2 (10)	4.0–29.0	0.90 (9)
Chaminade High School	37°00′16″N, 121°58′41″W; 100 m	10.0 ± 0.3 (6)	9.0–11.0	1.00 (6)
KOA Kampground	36°55′35″N, 121°50′35″W; 50 m	25.0 ± 6.4 (7)	4.7–53.0	0.43 (3)

[1]All plots were located in Santa Cruz County, CA.
[2]Approximate latitude, longitude and elevation of each plot.
[3]Mean lesion length in mm ± the standard error (n = number of trees inoculated).
[4]The shortest and longest lesions recorded on inoculated trees.
[5]The proportion of trees with mean lesion lengths shorter than 18 mm; the number of trees in this category is shown in parentheses.

3.2 Tests for susceptibility of trees manifesting disease remission

Fifty-five trees in five plots that manifested disease remission were selected for inoculation (Table 3). In four of the five plots, mean lesion lengths ranged from 7.8 ± 1.7 to 11.7 ± 2.5, and ≥90% of inoculated trees developed lesions shorter than 18 mm (Table 3), an approximate threshold for resistance (Gordon et al. 1998b). Results were more variable for trees in the fifth plot (KOA Kampground), which had mean lesion lengths ranging from 4.7 to 53.0 mm (n = 7); three trees (43%) had mean lesion lengths <18 mm. There were no obvious features of this site that might explain why more trees remained susceptible to pitch canker than in the other four plots. Across all five plots, 89% of trees (49/55) fell into the resistant category, as compared with 58% (93/160 old area trees) of trees randomly selected with respect to their disease status. If the latter is taken as the percentage of remission trees expected to be resistant, a chi square test shows the observed number to differ significantly from expectations ($p = 0.0024$).

4 Discussion

Where forest trees are exposed to an exotic pathogen, death or reduced productivity of susceptible individuals should enhance the relative contribution of less-affected trees to subsequent seed crops, thereby increasing the frequency of relatively resistant individuals in a population over time. Such a process of natural selection has presumably contributed to development of the relatively stable relationships commonly observed in native forests, where severe disease tends to be rare (Gordon et al. 2003). However, the transition from a population prone to severe damage to one that is less affected could be problematic for tree species with very long generation times, as a result of which significant shifts in allele frequency might require decades or even centuries. Thus, some more immediate defensive response(s) may be essential to allow inherently susceptible trees to persist while the slower process of genetic adaptation moves populations toward a more balanced relationship with a new microbial associate. One physiological mechanism by which plants can respond rapidly to pathogens is known as induced resistance, which may be manifested locally, in proximity to infected tissue, or systemically (Hammerschmidt 1999; Eyles et al. 2010). Resistance can be induced by strong or weak pathogens, and various abiotic factors, with the result that a plant becomes more resistant to subsequent challenge by an otherwise aggressive pathogen. Systemic induced resistance (SIR) has been documented to occur in coniferous trees in response to chemical inducers (Reglinski et al. 1998; Zeneli et al. 2006) and infection by pathogenic microbes (Krokene et al. 2001; Bonello et al. 2006), but no previous reports have established the importance of this physiological response under natural conditions. Our findings support the view that SIR is operative in *P. radiata*, and that this phenomenon is reducing the impact of pitch canker in California.

Tip dieback is a typical symptom of pitch canker and occurs where a lesion develops to the point of girdling a branch, but pathogen growth axially toward the main stem of the tree is usually quite limited (Gordon et al. 2001). Thus, severe damage from pitch canker requires repeated infections, each of which individually does relatively little damage. Whether or not disease progresses to the point of killing a tree, either directly or in conjunction with other forms of abiotic or biotic stress, depends, in part, on the tree's inherent susceptibility (Gordon et al. 1998b; Storer et al. 1999; Roux et al. 2007). However, even trees that manifest severe disease may survive because existing infections become inactive and new infections either do not occur or fail to develop to the point of causing visible damage (i.e. symptoms). Limits on development of pitch canker infections are a common feature of this disease and have been associated with recovery in loblolly pine (*Pinus taeda*) (Dwinell et al. 1985). The absence of new infections, the other essential component of recovery, may reflect the operation of various limiting factors such as availability of inoculum and the occurrence of suitable wounds under conditions conducive to infection (Sakamoto and Gordon 2007; Inman et al. 2008). Some combination of these factors may explain recovery from pitch canker in *P. taeda* and *P. radiata*. However, it is also possible that trees in remission have an elevated level of resistance to pitch canker, due to SIR.

In our tests of trees in remission, inoculum was delivered to an infection court under conditions known to be conducive to infection (Gordon et al. 1998a,b; Storer et al. 1999; Schmale and Gordon 2003; Sakamoto and Gordon 2007) thus eliminating the potential limiting factors described above. Fifty-five trees were tested in this way and 49 (89%) appeared resistant (mean lesion length < 18 mm), whereas only 38 and 58% of trees in new and old areas, respectively, could be placed in this category (n = 160 in both cases). Thus, disease remission is clearly associated with a higher level of resistance to pitch canker. For the six remission trees (11%) that did not appear to be resistant to pitch canker, factors other than host susceptibility were presumably responsible for the absence of disease.

Further support for the operation of SIR derives from the fact that trees in areas where pitch canker was well established were significantly less susceptible to the disease than trees in areas where the disease was a more recent occurrence. This is consistent with elevated resistance in trees with a longer period of exposure to the pitch canker pathogen. Furthermore, trees in new areas became more resistant over time, as indicated by the development of significantly shorter lesions in 2004 than when first inoculated in 2002. Although this temporal effect was evident in new areas, it was not detectable in old areas, which suggests that the maximal effect of SIR in these stands had already occurred.

In summary, three independent lines of evidence suggest that populations of *P. radiata* become more resistant to pitch canker over time as a consequence of exposure to *G. circinata*: (i) trees in areas where pitch canker is well established tend to sustain shorter lesions than trees in areas where the disease is a recent occurrence, (ii) most trees in remission were shown to be resistant to pitch canker based on their response to inoculations, and (iii) trees in new areas were less susceptible in 2004 than in 2002. Thus, it appears that *P. radiata* populations are capable of adapting to an exotic pathogen, at least in part, through the agency of SIR. More general operation of SIR could help to explain the evolutionary success of long-lived perennials that have historical associations with parasites manifesting generation times that are orders of magnitude shorter than that of their host trees.

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References

Anagnostakis, S.L., 1987: Chestnut blight – the classical problem of an introduced pathogen. Mycology **79,** 23–37.

Bonello, P.; Gordon, T.R.; Storer, A.J., 2001: Systemic induced resistance in Monterey pine. For. Pathol. **31,** 99–106.

Bonello, P.; Gordon, T.R.; Herms, D.A.; Wood, D.L.; Erbilgin, N., 2006: Nature and ecological implications of pathogen-induced systemic resistance in conifers: a novel hypothesis. Physiol. Mol. Plant Pathol. **68,** 95–104.

Burdon, J.J.; Thrall, P.H.; Ericson, L., 2006: The current and future dynamics of disease in plant communities. Annu. Rev. Phytopathol. **44,** 19–39.

Conover, W.J., 1999: Practical Nonparametric Statistics. New York: J. Wiley & Sons, Inc.

Dwinell, L.D.; Barrows-Broaddus, J.B.; Kuhlman, E.G., 1985: Pitch canker: a disease complex of southern pines. Plant Dis. **69,** 270–276.

Eyles, A.; Bonello, P.; Ganley, R.; Mohammed, C., 2010: Induced resistance to pests and pathogens in trees. New Phytol. **185,** 893–908.

Gordon, T.R., 2006: Pitch canker disease of pines. Phytopathology **96,** 657–659.

Gordon, T.R.; Okamoto, D.; Storer, A.J., 1998a: Susceptibility of five landscape pines to pitch canker disease, caused by *Fusarium subglutinans* f. sp. *pini*. HortScience **33,** 868–871.

Gordon, T.R.; Wikler, K.R.; Clark, S.L.; Okamoto, D.; Storer, A.J.; Bonello, P., 1998b: Resistance to pitch canker disease, caused by *Fusarium subglutinans* f. sp. *pini*, in Monterey pine (*Pinus radiata*). Plant Pathol. **47,** 706–711.

Gordon, T.R.; Storer, A.J.; Wood, D.L., 2001: The pitch canker epidemic in California. Plant Dis. **85,** 1128–1139.

Gordon, T.R.; Aegerter, B.J.; Friel, C.J., 2003: Stem canker diseases. In: Encyclopedia of Forest Sciences. Ed. by Evans, J.; Burley, J.; Youngquist, J. London: Elsevier, pp. 281–286.

Hammerschmidt, R., 1999: Induced disease resistance: how do induced plants stop pathogens? Physiol. Mol. Plant Pathol. **55,** 77–84.

Hepting, G.H.; Roth, E.R., 1946: Pitch canker, a new disease of some southern pines. J. For. **44,** 742–744.

Inman, A.R.; Kirkpatrick, S.C.; Gordon, T.R.; Shaw, D.V., 2008: Limiting effects of low temperature on growth and spore germination in *Gibberella circinata*, the cause of pitch canker in pine species. Plant Dis. **92,** 542–545.

Krokene, P.; Solheim, H.; Christiansen, E., 2001: Induction of disease resistance in Norway spruce (*Picea abies*) by necrotizing fungi. Plant Pathol. **50,** 230–233.

van Mantgem, P.J., 2004: Effects of an introduced pathogen and fire exclusion on the demography of sugar pine. Ecol. Appl. **14,** 1590–1602.

McCain, A.H.; Koehler, C.S.; Tjosvold, S.A., 1987: Pitch canker threatens California pines. Calif. Agric. **41,** 22–23.

Reglinski, T.; Stavely, F.J.L.; Taylor, J.T., 1998: Induction of phenylalanine ammonia lyase activity and control of *Sphaeropsis sapinea* infection in *Pinus radiata* by 5-chlorosalicylic acid. Eur. J. For. Pathol. **28,** 153–158.

Roux, J.; Eisenberg, B.; Kanzler, A.; Nel, A.; Coetzee, V.; Kietzka, E.; Wingfield, M.J., 2007: Testing of selected South African Pinus hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. New Forest. **33,** 109–123.

Sakamoto, J.M.; Gordon, T.R., 2007: Factors influencing infection of mechanical wounds by *Fusarium circinatum* on Monterey pines (*Pinus radiata*). Can. Entomol. **139,** 864–871.

Schmale, D.G.; Gordon, T.R., 2003: Variation in susceptibility to pitch canker disease, caused by *Fusarium circinatum*, in native stands of *Pinus muricata*. Plant Pathol. **52,** 720–725.

Sokal, R.R.; Rohlf, F.J., 1995: Biometry. New York: W.H. Freeman & Co.

Storer, A.J.; Bonello, P.; Gordon, T.R.; Wood, D.L., 1999: Evidence of resistance to the pitch canker pathogen (*Fusarium circinatum*) in native stands of Monterey pine (*Pinus radiata*). For. Sci. **45,** 500–505.

Storer, A.J.; Wood, D.L.; Gordon, T.R., 2002: The epidemiology of pitch canker in California. For. Sci. **48,** 694–700.

Wikler, K.; Storer, A.J.; Newman, W.; Gordon, T.R.; Wood, D.L., 2003: The dynamics of an introduced pathogen in a native Monterey pine (*Pinus radiata*) forest. For. Ecol. Manage. **179,** 209–221.

Zeneli, G.; Krokene, P.; Christiansen, E.; Krekling, T.; Gershenzon, J., 2006: Methyl jasmonate treatment of large Norway spruce (*Picea abies*) trees increases the accumulation of terpenoid resin components and protects against infection by *Ceratocystis polonica*, a bark beetle-associated fungus. Tree Physiol. **26,** 977–988.