

The role of *Pityophthorus* spp. as vectors of pitch canker affecting *Pinus radiata*

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Abstract—The fungus *Fusarium circinatum* Nirenberg and O'Donnell (Hypocreaceae) causes pitch canker, a disease affecting pines worldwide. In California, many native insect species have been implicated in transmission of *F. circinatum*. This study showed that two twig beetle species, *Pityophthorus setosus* Blackman and *Pityophthorus carmeli* Swaine (Coleoptera: Curculionidae: Corthylini), can make wounds on healthy Monterey pine (*Pinus radiata* D. Don (Pinaceae)) branches that are suitable for infection by the pitch canker pathogen. Because these two species are not known to engage in maturation feeding and the observed wounds were not associated with tunneling, we hypothesize that the wounds reflect “exploratory tasting” to assess the suitability of the substrate for colonization. This behavior would help to explain how twig beetles can serve as wounding agents on healthy host branches, which are not amenable to colonization by these insects. We tested two specific hypotheses: (1) two native species of *Pityophthorus* can create wounds on *F. circinatum*-contaminated trees that are sufficient for development of disease; and (2) the efficiency with which *F. circinatum* infects beetle wounds is affected by relative humidity. Under growth-chamber conditions, both *Pityophthorus* species indulged in exploratory behavior that caused wounds suitable for development of pitch canker. Field experiments did not confirm a significant effect of beetle activity on infection frequency, perhaps because of an overall low infection rate due to low temperatures. Experiments conducted under controlled conditions documented a significant effect of relative humidity on the success rate of twig beetle-initiated infections.

Résumé—Le champignon *Fusarium circinatum* Nirenberg et O'Donnell (Hypocreaceae) est responsable du chancre poisseux, une maladie qui affecte les pins à l'échelle planétaire. En Californie, plusieurs espèces d'insectes indigènes ont été reliées à la transmission de *F. circinatum*. Notre étude montre que deux scolytes des brindilles, *Pityophthorus setosus* Blackman et *Pityophthorus carmeli* Swaine (Coleoptera: Curculionidae: Corthylini), peuvent causer sur les branches de pins de Monterey (*Pinus radiata* D. Don (Pinaceae)) saines des blessures propres à permettre l'infection par le pathogène du chancre poisseux. Parce que ces deux espèces ne sont pas connues pour faire de l'alimentation de maturation et que les blessures observées ne sont pas associées à des galeries, notre hypothèse veut que ces blessures représentent des évaluations gustatives exploratoires de la convenance du substrat pour la colonisation. Ce comportement permettrait d'expliquer comment les scolytes des brindilles peuvent servir d'agents vulnérants sur les branches saines de l'hôte qui ne sont pas convenables pour la colonisation par ces insectes. Nous testons deux hypothèses spécifiques: (1) les deux espèces indigènes de *Pityophthorus* peuvent causer sur des arbres contaminés par *F. circinatum* des blessures suffisantes pour le développement de la maladie et (2) l'efficacité de l'infection à *F. circinatum* reliée aux blessures faites par les coléoptères est affectée par l'humidité relative. Dans des conditions de chambre de croissance, les deux espèces de *Pityophthorus* ont un comportement exploratoire qui cause des

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blesures suffisantes pour le développement du chancre poisseux. Des expériences de terrain ne confirment cependant pas l'existence d'un effet significatif de l'activité des coléoptères sur la fréquence des infections, peut-être à cause d'un taux global d'infection bas dû aux basses températures. Des expériences menées en conditions contrôlées indiquent un effet significatif de l'humidité relative sur le taux de succès des infections reliées aux scolytes des brindilles.

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Introduction

Pitch canker, caused by the fungus *Fusarium circinatum* Nirenberg and O'Donnell (Hypocreaceae), is a disease affecting numerous pine (*Pinus* L.) species (Pinaceae) (Gordon *et al.* 2001) and Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco (Pinaceae)) (Gordon *et al.* 2006). The disease is of global concern, especially for countries that maintain plantations of Monterey pine (*Pinus radiata* D. Don; also known as radiata pine) for lumber and wood products (Gordon *et al.* 2001). In California, where pitch canker was discovered in 1986 (McCain *et al.* 1987), native stands of Monterey pine and bishop pine (*Pinus muricata* D. Don) are threatened by the disease.

Several studies in California have implicated native insect species in the transmission of *F. circinatum* between pine hosts (Fox *et al.* 1990, 1991; Hoover *et al.* 1995, 1996; Storer *et al.* 2004). The engraver beetles *Ips mexicanus* Hopkins (= *Pseudips mexicanus* Hopkins) and *Ips paraconfusus* Lanier (Coleoptera: Curculionidae: Ipini) were abundant in pitch canker-infested areas, and pitch canker symptoms developed when *F. circinatum*-contaminated *I. paraconfusus* beetles were either artificially inserted into plant material or confined on logging debris or on the main stem of live seedlings (Fox *et al.* 1990, 1991). Hoover *et al.* (1995, 1996) demonstrated that both the dry twig and cone beetle, *Ernobius punctulatus* (LeConte) (Coleoptera: Anobiidae: Ernobiini), and the Monterey pine cone beetle, *Conophthorus radiatae* Hopkins (Coleoptera: Curculionidae: Corthylini), were associated with *F. circinatum* and that *C. radiatae* was capable of transmitting the pathogen. McNee *et al.* (2002) showed that twig beetles, *Pityophthorus* Eichoff (Coleoptera: Curculionidae: Corthylini), emerged predominantly from *F. circinatum*-infected branch tips with yellow and (or) red needles rather than from asymptomatic, green-needled branch tips. Storer *et al.* (2004) conducted several experiments in which *F. circinatum*-contaminated *Pityophthorus setosus* Blackman could transfer infectious *F. circinatum* propagules when confined to branches

of *P. radiata* and that pitch canker incidence was higher on potted trees baited with *Pityophthorus*-specific pheromones.

Collectively, these studies have satisfied Leach's postulates (Leach 1940) and thus support a role for various insects, including twig beetles, as vectors of the pitch canker pathogen. However, it remains unclear how the life history of twig beetles would provide opportunities for transmission of the pitch canker pathogen under natural conditions. *Pityophthorus* spp. breed in dead or dying branches and thus can come in contact with *F. circinatum* when they colonize a pitch canker-killed branch (Gordon *et al.* 2001). Progeny that emerge from such branches may carry *F. circinatum* spores, but *Pityophthorus* spp. are not known to have a maturation feeding stage. If they were to preferentially seek out a declining branch in which to breed, this would not allow for transmission of the pathogen to a healthy branch. Hence, it has been hypothesized that *Pityophthorus* spp., which appear to be incapable of locating weakened branches prior to landing (Bonello *et al.* 2001), will land on healthy branches and create wounds in the process of assessing the suitability of the substrate for colonization through "exploratory tasting" (Gordon *et al.* 2001; Storer *et al.* 2004). However, it has yet to be established that unconstrained *Pityophthorus* spp. will make such wounds on healthy branches. Consequently, the first objective of the present study was to determine whether two *Pityophthorus* species were capable of creating wounds suitable for infection by *F. circinatum* when allowed freedom of movement on trees under controlled conditions. We also conducted a field experiment to determine whether, through the use of pheromone attractants, we could elevate the activity of twig beetles sufficiently to allow us to document a corresponding increase in the incidence of pitch canker.

The second objective of this study was to determine the effect of environmental conditions on infection by the pitch canker pathogen. Previous work has documented greater disease severity on the coast than in more inland locations

(≥ 1.5 km) (Wikler *et al.* 2003). This may reflect an influence of atmospheric moisture because if wounds dry too quickly, *F. circinatum* spores may not germinate in time to infect the wounded tissue. Thus, the greater frequency and duration of fog near the coast may allow for a higher rate of infection. However, experiments conducted under both controlled conditions and in the field showed no significant effect of relative humidity on infection frequency (Sakamoto and Gordon 2006). The wounds in this case were mechanically induced and may have been large and deep enough to minimize the effect of atmospheric conditions on the infection process. Alternatively, wounds created by insects in the process of exploratory tasting would be much smaller and perhaps more prone to drying; hence, they would be subject to limitations imposed by ambient conditions. To test this hypothesis we conducted experiments under controlled conditions to determine whether the frequency of twig beetle-induced infections was influenced by postinoculation relative humidity.

Materials and methods

Inoculum

All spore suspensions used for inoculations were prepared from *F. circinatum* isolate GL17 (= Fsp17), which is available on request from T.R. Gordon, Department of Plant Pathology, University of California at Davis. This isolate was originally obtained from an infected Monterey pine in Pebble Beach, California. Spore suspensions were prepared by adding sterile 0.5% KCl to fully colonized plates of potato dextrose agar and lightly scraping mycelium with a sterile bent glass rod (Gordon *et al.* 1998). The suspension was passed through double layers of cheesecloth and quantified using a hemacytometer. The desired concentration was achieved by dilution with sterile 0.5% KCl solution. An additional dilution to obtain 1500 spores/mL (target dose 75 spores/50 μ L) was made and 50 μ L was spread onto each of five potato dextrose agar plates (a total of 85 plates for these experiments) to confirm viability of the inoculum. After 2–3 days at room temperature, the number of germinated spores was recorded and percent viability was determined to be $84.2 \pm 1.67\%$ (mean \pm SEM) of the target dose.

Cages

For growth-chamber experiments, two cages were designed to contain *Pityophthorus* spp. while allowing them to move freely in proximity

to trees potted in 9 L containers (inner diameter 21 cm). The frame of each cage was constructed from 1.9 cm diameter PVC pipes and covered on the sides and top by a fine-mesh material (No-Thrips[®] No. 7261B, hole opening 0.0059 in. \times 0.0059 in. (1 in. = 25.4 mm), mesh size 81 mm \times 81 mm; BioQuip Products, Rancho Dominguez, California). The cage was sealed with duct tape to a tarpaulin that functioned as the floor of the cage. A door was fabricated out of one wall for access into the cages during experiments. Pheromone baits attractive to *Pityophthorus* spp. were suspended from the center top of the PVC frame in each cage. Baits consisted of 5 μ L of active compound in 1.5 mL microfuge tubes that were closed and pierced once through the cap with a heated No. 2 insect pin to release the pheromone (Dallara *et al.* 2000). *E*-(+/-)-pityol (4.58 mg) was used in experiments with *P. setosus*, and both pityol and *E*-(-)-conophthorin (4.36 mg) were used for experiments with *Pityophthorus carmeli* Swaine. After each experiment the cages were decontaminated with 1.0% sodium hypochlorite.

Beetles

Beetles were collected from wild populations in Pebble Beach, California, using eight-unit Lindgren funnel traps (Lindgren 1983) baited with pityol to capture *P. setosus* or pityol and conophthorin to capture *P. carmeli* (Dallara *et al.* 2000). Beetle samples were kept on ice until they were sent to the laboratory, where they were maintained at 4 °C for up to 14 days prior to their use in experiments. Beetles were immobilized in a glass Petri plate suspended on a bed of ice for viewing under a dissecting microscope, and were identified to species, based on criteria specified by Bright (1981). Sex determination was not performed, although typical trap-catch sex ratios observed previously using these baits had been male-biased for *P. setosus* (85.5% male : 14.5% female, $n = 20$ and 549, respectively) and female-biased for *P. carmeli* (11.0% male : 89.0% female, $n = 3$ and 275, respectively) (Sakamoto 2004). A subset of beetles was plated on a *Fusarium*-selective medium to assay for the presence of *F. circinatum* (Gordon *et al.* 1998).

Rating of lesions and confirmation of the presence of *F. circinatum*

We confirmed the presence of *F. circinatum* in plant material following the methods of Gordon *et al.* (1998). Briefly, each tree or branch was visually observed for obvious

pitching or darkening of the bark. The bark was removed from the entire branch or suspected lesion site and cut into 1.6 cm pieces, which were surface-disinfected, and plated onto *Fusarium*-selective medium (Gordon *et al.* 1998).

Role of two *Pityophthorus* species as wounding agents under controlled conditions

Pityophthorus setosus and *P. carmeli* were investigated as potential wounding agents under controlled conditions. Eighteen 2- to 3-year-old potted *P. radiata* trees in 9 L pots (approximately 1 m in height, main stem diameter between 1.3 and 2 cm) were sprayed to runoff with a suspension of *F. circinatum* (10^7 spores/mL). Inoculum was allowed to dry on trees overnight in a growth chamber set to a diurnal cycle of 16 h of light at 25 °C and 8 h of dark at 18 °C (to simulate a day–night cycle). Nine trees were placed inside a screened cage and nine trees remained outside the cage. One branch tip (approximately 7.6 cm long) on each tree was randomly chosen and enclosed in a bag made of No-Thrips[®] material to protect it from beetle activity. After beetles were removed, the No-Thrips[®] bag was removed and the branches were wounded using a 1.6 mm diameter drill bit at 5 points (approximately 1.6 cm apart). These mechanically wounded sites served as positive controls for symptom development. Data loggers were placed inside and outside the cage from the beginning of the experiment to record temperature and relative humidity (RH). Ambient growth-chamber RH ranged from 63.3% to 82.9%

Inside the cage, ≥ 500 beetles were released from Petri dishes placed at the base of the trees, which were surrounded by a tree skirt fashioned from No-Thrips[®] material to prevent beetles from falling into the potting mix. After 48 h, all beetles were removed from the cage. Each tree was wounded at the protected positive-control site and loosely covered with plastic to maintain high RH for 24 h (confirmed to be 100% by data-logger measurements). Thereafter, trees were removed from the cage, placed with control trees that had not been exposed to beetle activity, and allowed to incubate for 6 weeks in the growth chamber. All trees were then examined for symptoms of pitch canker. A tree was visually identified as infected if it exhibited pitching, yellowing needles, and resinous lesions. Infection by *F. circinatum* was confirmed as the cause of each lesion by culturing excised

lesions on *Fusarium*-selective medium (Gordon *et al.* 1998). Infection frequency (percent diseased) was calculated as the number of infected trees in each treatment divided by the total number of trees in that treatment.

This experiment was conducted twice with *P. setosus* (initiated on 11 December 2000 and 2 May 2001) and once with *P. carmeli* (initiated on 31 March 2003). In the *P. carmeli* experiment, the number of trees used was reduced to eight per treatment group. The results were analyzed using Fisher's exact test (Sokal and Rohlf 2003). Trial homogeneity of variance was tested using the nonparametric squared ranks test (StatsDirect[®], StatsDirect Limited, 11 Gresham Way, Altrincham, Cheshire M33 3UY, United Kingdom). Exact confidence intervals were calculated from the binomial distribution (Steel *et al.* 1997).

Effect of RH on infection of beetle-created wounds in a growth chamber

This experiment was conducted to determine whether high RH significantly increased frequencies of infection of beetle-created wounds by *F. circinatum* and to confirm the results of the experiments with wounding agents described above. In both trials, eight different potted trees were sprayed to runoff with a suspension of *F. circinatum* spores (10^7 /mL) and allowed to dry overnight in a growth chamber set as described above. All plants were placed in one No-Thrips[®] cage (as described above) and exposed to approximately 500 *P. setosus*. On each tree, a randomly chosen branch that served as a mechanical-wounding site (positive control) was protected from *P. setosus* activity with No-Thrips[®] material. After 48 h, trees were removed from the beetle enclosure and four were placed in individual plastic bags to maintain high RH for an additional 24 h. The other four were left uncovered. Data loggers were placed inside the bags and in the growth chamber to record RH and temperature. RH within bags was measured at 100% and ambient growth-chamber humidity was measured within the range 49.4%–82.9%. After 6 weeks, bark was removed from each tree and the number of lesions was recorded. *Fusarium circinatum* was confirmed as the cause of the lesion as described above. The experiment was conducted twice, with initiation on 7 August 2002 and 14 August 2002. Given the small sample size, the differences between treatments were analyzed with the nonparametric Mann–

Whitney *U* test (Sokal and Rohlf 2003). Trial homogeneity of variance was tested using the nonparametric squared ranks test (StatsDirect®).

Field test for beetle-mediated infections

In this experiment the potential for *P. setosus* to act as a wounding agent or vector under natural conditions was investigated. Each trial included three pairs of field plots (baited and unbaited) in Pebble Beach, California. A plot consisted of a stand of young trees (0.6–1.2 m in height) within a circular area 3.1 m in diameter. All plots were placed in areas in which previous trap catches of *P. setosus* and *P. carmeli* (Sakamoto 2004) had been abundant and disease incidence was low to moderate. In each plot, 10 trees were selected and randomly designated as *F. circinatum*-contaminated or non-*F. circinatum*-contaminated. Each plot was at least 10 m from the edge of the nearest adjacent plot. The five *F. circinatum*-contaminated trees were sprayed to runoff with 10^7 spores/mL. During the application, a poster-board enclosure (0.91 m × 1.2 m) was placed around trees to minimize drift of spores outside the target area. At the center of each baited plot, a microfuge tube with racemic *E*-(±)-pityol (4.58 mg) was secured to a 1.2 m high wooden stake to serve as a lure. The bait was intended to draw insects to the trees within the radius but was not secured to any single tree. *Fusarium circinatum*-contaminated trees in baited plots provided a means of detecting infections that might occur when an insect, attracted by the pheromone, landed on a branch where inoculum was already present. Non-contaminated trees in baited plots were used to determine whether infection of healthy trees might result from insect-mediated wounding in the presence of naturally occurring inoculum, either on the insect or on the branch. Unbaited contaminated and non-contaminated trees were used as controls.

Each baited plot was paired with a nonbaited plot that was set up in an identical manner, except that a blank tube was used instead of one containing pityol. Pheromone baits were replaced 7 weeks after the experiment was initiated. Lindgren funnel traps baited with the same material as in the baited plot were used to estimate beetle activity in the plots 0 and 7 weeks after the experiment was initiated. Traps were placed on nearby trees (approximately 10 m from the center to avoid cross-interference with bait in the center of the plot). At weeks 0 and 7, trap catches were quantified

on each of 3 successive days. The number of *P. setosus* per plot trapped each day across 3 days and three plots was 443 ± 13.9 (mean \pm SEM; $n = 12$ traps) in week 0 and 3861 ± 74.2 ($n = 12$ traps) in week 7. At the conclusion of the experiment (20 weeks after initiation) the number of infected trees per stand and the number of infection points (lesions and symptomatic areas) per tree were recorded. Infection status of lesions was confirmed as described above. Ambient RH when experiments were initiated was within the range 62.8%–93.4%. Ambient temperature ranged from 7.3 to 14.3 °C. The experiment was conducted twice in February 2003. Data were analyzed using Fisher's exact test (Sokal and Rohlf 2003). Trial homogeneity was tested using the nonparametric squared ranks test (StatsDirect). Exact confidence intervals were calculated from the binomial distribution (Steel *et al.* 1997)

Results

Role of two *Pityophthorus* species as wounding agents under controlled conditions

In preliminary experiments *P. setosus* was observed creating wounds on branches of trees under controlled conditions. Some wounds had fresh resin exuding from them, and in at least one case, a beetle was trapped with its head in the resin. When the full experiment was conducted, most infections were observed to be associated with visible beetle-caused wounds. Infections also occurred where mechanical wounds had been made (controls). These observations suggest that in the presence of viable inoculum, injuries can serve as infection courts.

Variances for trials using *P. setosus* were found to be homogeneous (squared ranks test, $z = 0.861$, two-tailed $P = 0.390$). Data for the two trials were pooled to increase the overall power of the analysis. Significantly more of the trees exposed to beetles were infected with *F. circinatum* (88.9%, $n = 18$, 95% binomial CI = 65%–99%) than of the trees not exposed to beetles (5.6%, $n = 18$, 95% binomial CI = 0%–27%) (Fisher's exact test, two-sided $P < 0.0001$). Lesions were observed at all 36 mechanical wounding sites, and the presence of *F. circinatum* was confirmed as described above. This confirmed the viability of the inoculum on trees that were otherwise not infected. The one infected tree not exposed to beetle activity was found to have mealy bugs (Hemiptera: Pseudococcidae) at the infection sites. It is

probable that wounding caused by the mealy bugs provided infection courts for *F. circinatum*. To prevent this from occurring in subsequent growth-chamber experiments, 2 weeks prior to initiation of an experiment all trees were treated with one of the following insecticides: Talstar, Provado, or Enstar® II.

In the one trial with *P. carmeli*, seven of eight beetle-exposed trees (87.5%, $n = 8$, 95% binomial CI = 47%–99.7%) were infected with *F. circinatum*, significantly more than the two of eight trees (25%, $n = 8$, 95% binomial CI = 3%–65%) not exposed to beetles that became infected (Fisher's exact test, two-sided $P = 0.04$). All 16 control branches with the mechanical-wound sites were found to be infected (as described above), confirming the viability of the inoculum on the non-infected trees.

Effect of RH on infection of beetle-created wounds in the growth chamber

Data from both trials were not normally distributed and were therefore analyzed using the nonparametric Mann–Whitney U test combined with a corresponding nonparametric homogeneity of variances (squared ranks) test. Data from both trials were combined after we determined that variances were homogeneous (squared ranks test, $z = -1.14$, two tailed $P = 0.253$). Trees exposed to *P. setosus* and subsequently enclosed in plastic had significantly more lesions ($3.37 \pm 0.596/\text{tree}$; mean \pm SEM) than trees kept at ambient RH in the growth chamber ($1.87 \pm 0.295/\text{tree}$) (Mann–Whitney U test, $P = 0.017$). Tests on all 16 positive-control branches (mechanical-wound sites) confirmed the presence of *F. circinatum* (as described above), documenting the viability of the inoculum on non-infected trees.

Field test for beetle-mediated infections

Since variances for trials were heterogeneous (square ranks test, $z = 6.38$, two-tailed $P < 0.0001$), data for the two trials were analyzed separately. In trial 1, 20% of 15 *F. circinatum*-contaminated trees in the baited plots became infected, but this was not significantly different from the 6.67% ($n = 15$) of *F. circinatum*-contaminated trees in the nonbaited plots that became infected (Fisher's exact test, two-sided $P = 0.60$). In trial 2, 6.67% ($n = 15$) of *F. circinatum*-contaminated trees in the baited plots became infected, and this was not significantly different from the 0% of 15 *F. circinatum*-contaminated trees in nonbaited

plots that became infected (Fisher's exact test, two-sided $P > 0.99$) (Fig. 3). In both trials, none of the control trees (not contaminated with *F. circinatum*) became infected in either baited or nonbaited plots.

Discussion

Close relationships between bark beetles and fungal pathogens are not uncommon. Ambrosia beetles carry their ophiostomatoid fungi from one tree to another in specialized mycangial invaginations as food and protection against tree defenses (Farrell *et al.* 2001). Similarly, phloem-feeding bark beetles transmit ophiostomatoid fungi such as the Dutch elm disease pathogens *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* and several blue stain fungi (Brasier 1996; Guérard *et al.* 2000). In North America, the two Dutch elm disease vectors are the native elm bark beetle, *Hylurgopinus rufipes* (Eichhoff), and the European elm bark beetle, *Scolytus multistriatus* (Marsham) (Coleoptera: Curculionidae: Scolytinae) (Lanier 1982). Both species breed in weakened or diseased elms and when they emerge, feed in twig crotches on healthy elms, thereby inoculating fresh material (Lanier 1982). In contrast, twig beetles on Monterey pines are thought not to feed on healthy tissue, but rather to feed and breed in shade-suppressed or pitch canker-afflicted branches (Gordon *et al.* 2001). Further, *Pityophthorus* spp. were not drawn to host volatiles released from cut branches (Bonello *et al.* 2001). We therefore hypothesized that twig beetles engaged in postlanding assessment of substrate suitability through exploratory tasting.

The results of experiments conducted under growth-chamber conditions demonstrate that *Pityophthorus* spp. can induce wounds suitable for infection by *F. circinatum* on healthy branches. Furthermore, wounds were induced by insects that were allowed freedom of movement over the surfaces of multiple trees. This confirms and extends the results of previous studies showing that infections can be initiated by infested twig beetles strictly confined to branches (Dallara 1997; Storer *et al.* 2004). In aggregate, the data are consistent with the hypothesis that *Pityophthorus* spp. can introduce *F. circinatum* to healthy branches, even branches that are not subjected to the tunneling activity associated with colonization and breeding.

Under field conditions, trees artificially contaminated with *F. circinatum* in baited plots

sustained more infections than *F. circinatum*-contaminated trees in unbaited plots, but this difference was not statistically significant. The lack of a significant treatment effect is likely not due to insufficient activity of *P. setosus*. Within 30 min after baits were set, large numbers of *P. setosus* were observed aggregating around release points and the surrounding trees. Furthermore, trap catches confirmed moderate to high activity at the beginning and middle of the experiment (weeks 0 and 7, respectively). On the other hand, it may be that relatively few *P. setosus*-created wounds became infected because of limitations imposed by environmental conditions. Recent work has shown that infection frequency is greatly reduced at temperatures ≤ 10 °C (Inman and Gordon 2005), and field temperatures as low as 7.3 °C were recorded during our field study. Thus, low temperatures during the experimental period may have dictated a low infection rate, making it difficult to resolve differences in effects between treatments. Although we did not observe a significant treatment effect within the time frame of our study, Storer *et al.* (2004) showed that over a 3 year period, potted trees were significantly more likely to become infected when baited with *P. setosus* pheromones than when unbaited.

Previous studies have shown a significant geographical effect on the severity of pitch canker on the Monterey Peninsula. Coastal plots of *P. radiata* are more severely affected by pitch canker than those located farther inland (Wikler *et al.* 2003). One plausible explanation for this effect is that the greater frequency and duration of fog near the coast facilitate infection by allowing superficial wounds to remain moist long enough for fungal propagules to germinate and become established. Consistent with this expectation, the present study documented a significant effect of RH on the frequency of infection of wounds created by *P. setosus* under controlled conditions. *Fusarium circinatum* infections associated with wounding by *P. setosus* may therefore be limited by climate in arid regions of the western United States of America.

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References

- Bonello, P., McNee, W.R., Storer, A.J., Wood, D.L., and Gordon, T.R. 2001. The role of olfactory stimuli in the location of weakened hosts by twig-infesting *Pityophthorus* spp. *Ecological Entomology*, **26**: 8–15.
- Brasier, C. 1996. New horizons in dutch elm disease control. Report on Forest Research, Forestry Commission, Edinburgh, United Kingdom. pp. 20–28.
- Bright, D.E. 1981. Taxonomic monograph of the genus *Pityophthorus* Eichhoff in North and Central America (Coleoptera: Scolytidae). *Memoirs of The Entomological Society of Canada* No. 118.
- Dallara, P.L. 1997. Studies on the distribution, interspecific relationships, host range, and chemical ecology of *Pityophthorus* spp. (Coleoptera: Scolytidae) and selected insectan associates, and their associations with *Fusarium subglutinans* f. sp. *pini* in central coastal California. Ph.D. dissertation, University of California, Berkeley, California.
- Dallara, P.L., Seybold, S.J., Meyer, H., Tolasch, T., Francke, W., and Wood, D.L. 2000. Semiochemicals from three species of *Pityophthorus* (Coleoptera: Scolytidae): identification and field response. *The Canadian Entomologist*, **132**: 889–906.
- Farrell, B.D., Sequeira, A.S., O'Meara, B.C., Normark, B.B., Chung, J.H., and Jordal, B.H. 2001. The evolution of agriculture in beetles (Curculinidae: Scolytinae and Platypodinae). *Evolution*, **55**: 2011–2027.
- Fox, J.W., Wood, D.L., and Koehler, C.S. 1990. Distribution and abundance of engraver beetles (Scolytidae: *Ips* spp.) on Monterey pines infected with pitch canker. *The Canadian Entomologist*, **122**: 1157–1166.
- Fox, J.W., Schultz, M.E., Gordon, T.R., and Wood, D.L. 1991. Engraver beetles (Scolytidae: *Ips* species) as vectors of the pitch canker fungus, *Fusarium subglutinans*. *The Canadian Entomologist*, **123**: 1355–1367.
- Gordon, T.R., Okamoto, D., Storer, A.J., and Wood, D.L. 1998. Susceptibility of five landscape pines to pitch canker disease, caused by *Fusarium subglutinans* f. sp. *pini*. *Hortscience*, **33**: 868–871.
- Gordon, T.R., Storer, A.J., and Wood, D.L. 2001. The pitch canker epidemic in California. *Plant Disease*, **85**: 1128–1139.
- Gordon, T.R., Kirkpatrick, S.C., Aegerter, B.J., Wood, D.L., and Storer, A.J. 2006. Susceptibility of Douglas-fir (*Pseudotsuga menziesii*) to pitch

- canker, caused by *Gibberella circinata* (anamorph = *Fusarium circinatum*). *Plant Pathology*, **55**: 231–237.
- Guérard, N., Dreyer, E., and Lieutier, F. 2000. Interactions between Scots pine, *Ips acuminatus* (Gyll.) and *Ophiostoma brunneo-ciliatum* (Math.): estimation of the critical thresholds of attack and inoculation densities and effects on hydraulic properties in the stem. *Annals of Forest Science*, **57**: 681–690.
- Hoover, K., Wood, D.L., Fox, J.W., and Bros, W.E. 1995. Quantitative and seasonal association of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini* with *Conophthorus radiatae* (Coleoptera: Scolytidae) and *Ernobius punctulatus* (Coleoptera: Anobiidae) which infest *Pinus radiata*. *The Canadian Entomologist*, **127**: 79–91.
- Hoover, K., Wood, D.L., Storer, A.J., Fox, J.W., and Bros, W.E. 1996. Transmission of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*, to Monterey pine, *Pinus radiata*, by cone- and twig-infesting beetles. *The Canadian Entomologist*, **128**: 981–994.
- Inman, A., and Gordon, T.R. 2005. Temperature as a potential barrier for the spread of pitch canker. *Phytopathology*, **95**: S46.
- Lanier, G.N. 1982. Dutch elm disease: a postscript. *Arnoldia*, **42**: 78–87.
- Leach, J.G. 1940. *Insect transmission of plant diseases*. McGraw-Hill Book Company, Inc., New York.
- Lindgren, B.S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *The Canadian Entomologist*, **115**: 299–302.
- McCain, A.H., Koehler, C.S., and Tjosvold, S.A. 1987. Pitch canker threatens California pines. *California Agriculture*, **41**: 22–23.
- McNee, W.R., Wood, D.L., Storer, A.J., and Gordon, T.R. 2002. Incidence of the pitch canker pathogen and associated insects in intact and chipped Monterey pine branches. *The Canadian Entomologist*, **134**: 47–58.
- Sakamoto, J.M. 2004. Exploring the roles of environmental factors and the twig beetle, *Pityophthorus* spp. (Coleoptera: Scolytidae) in the epidemiology of pitch canker of *Pinus radiata*. Ph.D. dissertation, University of California, Davis, California.
- Sakamoto, J.M., and Gordon, T.R. 2006. Factors influencing infection of mechanical wounds by *Fusarium circinatum* on Monterey pines (*Pinus radiata*). *Plant Pathology*, **55**: 130–136.
- Sokal, R.R., and Rohlf, F.J. 2003. *Biometry*. 3rd ed. W.H. Freeman and Company, New York.
- Steel, R.G.D., Torrie, J.H., and Dickey, D. 1997. *Principles and procedures of statistics: a biometrical approach*. 3rd ed. The McGraw-Hill Company, Inc., New York.
- Storer, A.J., Wood, D.L., and Gordon, T.R. 2004. Twig beetles, *Pityophthorus* spp. (Coleoptera: Scolytidae) as vectors of the pitch canker pathogen, *Fusarium circinatum* in California. *The Canadian Entomologist*, **136**: 685–693.
- Wikler, K., Storer, A.J., Newman, W., Gordon, T.R., and Wood, D.L. 2003. The dynamics of an introduced pathogen in a native Monterey pine (*Pinus radiata*) forest. *Forest Ecology and Management*, **179**: 209–221.