Incidence of the pitch canker pathogen and associated insects in intact and chipped Monterey pine branches

William R McNee,[1,2] David L Wood, Andrew J Storer[3]

Division of Insect Biology, University of California, Berkeley, California, United States 94720

Thomas R Gordon

Department of Plant Pathology, University of California, Davis, California, United States 95616

The Canadian Entomologist **134**: 47 – 58 (2002)

Abstract—In a 2-year study of Monterey pine, *Pinus radiata* D. Don (Pinaceae), infected with pitch canker, caused by *Fusarium circinatum* Nirenberg and O'Donnell, less than 2% of symptomatic branches with green foliage were colonized by twig beetles in the genus *Pityophthorus* Eichhoff (Coleoptera: Scolytidae), whereas approximately 50% of branches with yellow and red foliage were colonized. More *Pityophthorus* spp. emerged from yellow branches (mean ± SE = 12.1 ± 1.7 per 30 cm) than from red branches (6.9 ± 0.9) at an inland study site (Oakland) but, at a coastal site (Pebble Beach), the means were not significantly different (4.3 ± 0.6 and 3.8 ± 0.7). The mean phoresy rate of all emerging insects was higher at Pebble Beach (17.7 ± 0.6%) than at Oakland (5.3 ± 0.2%). At both sites, there was considerable temporal variation in the proportion of branches colonized by twig beetles, mean numbers of emerging twig beetles, and phoresy rates of emerging insects. Chipping branches reduced the emergence of *Pityophthorus* spp. and associates by approximately 95%, compared with emergence from intact branches. The pathogen was isolated from 1-year-old branches and chips in up to 68% of samples, but was only recovered from 3-year-old branches in 1 of 46 sampled. It is recommended that recent branch cuttings and chips originating from symptomatic trees not be transported to areas that are believed to be free of the disease.

McNee WR, Wood DL, Storer AJ, Gordon TR. 2002. Influence du chancre fusarien et des insectes associés dans des branches intactes et des copeaux de pins de Monterey. *The Canadian Entomologist* **134** : 47–58.

Résumé—Au cours d'une étude de 2 ans sur des pins de Monterey, *Pinus radiata* D. Don (Pinaceae), porteurs du chancre fusarien causé par *Fusarium circinatum* Nirenberg and O'Donnell, moins de 2 % des branches à feuillage vert porteuses de symptômes étaient colonisées par des scolytes des rameaux appartenant au genre *Pityophthorus* Eichhoff (Coleoptera : Scolytidae), alors qu'environ 50 % des branches à feuillage jaune ou rouge étaient infestées. Un plus grand nombre de *Pityophtorus* spp. ont émergé des branches jaunes (moyenne ± écart type = 12,1 ± 1,7 par 30 cm) que des branches rouges (6,9 ± 0,9) au cours d'une étude à un site à l'intérieur des terres (Oakland), mais à un site le long de la côte (Pebble Beach) les moyennes ne différaient pas significativement (4,3 ± 0,6 et 3,8 ± 0,7). Le taux moyen de phorésie de tous les insectes à l'émergence était plus élevé à Pebble Beach (17,7 ± 0,6 %)

[1] Corresponding author (e-mail: mcneeb@dnr.state.wi.us).
[2] Present address: Wisconsin Department of Natural Resources, 1125 North Military Avenue, Green Bay, Wisconsin, United States 54307.
[3] Present address: School of Forestry and Wood Products, Michigan Technological University, 1400 Townsend Drive, Houghton, Michigan, United States 49931.

qu'à Oakland (5,3 ± 0,2 %). Aux deux sites, il y avait une variation temporelle considérable de la proportion de branches colonisées par les scolytes des rameaux, du nombre moyen de scolytes à l'émergence et des taux de phorésie chez les insectes émergés. La taille des branches en copeaux réduisait d'environ 95 % l'émergence des *Pityophthorus* spp. et autres espèces associées comparativement à l'émergence dans les branches intactes. L'agent pathogène a pu être isolé à partir de branches et de copeaux de bois de 1 an dans 68 % des échantillons, mais n'a été trouvé dans les branches de 3 ans que dans1 échantillon sur 46. Nous recommandons d'éviter le transport de branches coupées et de copeaux provenant d'arbres porteurs de symptômes dans les zones d'où la maladie semble absente.

[Traduit par la Rédaction]

Introduction

Pitch canker, caused by the fungus *Fusarium circinatum* Nirenberg and O'Donnell (the asexual form of the ascomycete *Gibberella circinata* Nirenberg and O'Donnell) (Nirenberg and O'Donnell 1998), was first identified in California in 1986 from Monterey pine, *Pinus radiata* D. Don (Pinaceae), in Santa Cruz County (McCain *et al.* 1987). The disease is now found in all three native Monterey pine stands in California, and occurs from Mendocino County to San Diego County (Correll *et al.* 1991; Storer *et al.* 1994; Dallara *et al.* 1995). The San Francisco Bay area and the central coast have been most heavily affected, with a large number of dead and dying trees (Dallara *et al.* 1995). Symptoms of the disease include characteristic dead branch tips, resinosis, resin-soaked lesions in the xylem and phloem, aborted cones on infected branches, and resinous cankers on the main stem (McCain *et al.* 1987; Correll *et al.* 1991). Bark beetles may cause top-kill after much of the crown has been killed by the pathogen, and the entire tree may ultimately be killed by the pathogen and (or) bark beetles (Fox *et al.* 1990).

In California, pitch canker affects many other native pine species and Douglas-fir [*Pseudotsuga menziesii* (Mirbel)] Franco (Pinaceae) (McCain *et al.* 1987; Storer *et al.* 1997). Introduced pine species such as Aleppo pine (*Pinus halepensis* Mill.), Italian stone pine (*P. pinea* L.), and Canary Island pine (*P. canariensis* C. Smith) are also susceptible to the disease (McCain *et al.* 1987), although much less so than many native species (Gordon *et al.* 1998*a*). In the southeastern United States, the pathogen causes economic losses in pine plantations and seed orchards (Dwinell *et al.* 1985).

In California, *F. circinatum* is vectored into the branches, cones, and stems of susceptible trees by several genera of beetles (Correll *et al.* 1991; Fox *et al.* 1991; Hoover *et al.* 1996). Twig beetles in the genus *Pityophthorus* Eichhoff (Coleoptera: Scolytidae) have been demonstrated to vector the pathogen in the laboratory (Fox *et al.* 1991) and under field conditions (AJ Storer, DL Wood, TR Gordon, unpublished data). Twig beetles fly during most of the year along the central California coast (Dallara 1997), and they colonize recently killed or weakened branches and portions of the main stem between the larval galleries of other bark beetle species (Furniss and Carolin 1977). *Pityophthorus* spp. caused negligible economic damage in California prior to the introduction of pitch canker (Bright and Stark 1973), but as vectors of the pathogen, this genus has assumed an important role in the ecology of coastal pine forests (Storer *et al.* 1999).

Other important beetle genera are also known to carry the pathogen. Engraver beetles in the genus *Ips* DeGeer (Coleoptera: Scolytidae) colonize the main stem and larger branches of pines and are known to vector the pathogen (Fox *et al.* 1991). The dry twig beetle, *Ernobius punctulatus* Fall (Coleoptera: Anobiidae), colonizes dead branches and cones (Furniss and Carolin 1977; Ohmart 1981) and has been demonstrated to vector the pathogen (Hoover *et al.* 1996). Cylindrical bark beetles in

the genus *Lasconotus* Erichson (Coleoptera: Colydiidae) are predators that inhabit scolytid galleries (Furniss and Carolin 1977) and carry propagules of *F. circinatum* when emerged from diseased branches (Dallara *et al.* 1995). An association between spittlebug nymphs, *Aphrophora canadensis* Walley (Hemiptera: Cercopidae), and incidence of the disease in Monterey pine shoots has been demonstrated (Storer *et al.* 1998).

Recommended disease-management strategies include planting resistant trees or less-susceptible tree species (Storer *et al.* 1994; Gordon *et al.* 1998*a*, 1998*b*), reducing the amount of bark beetle breeding substrate, and careful disposal of infected material to avoid spreading the disease. To establish guidelines for the disposal of infected branches and branch chips, several studies were initiated in 1997 to (*i*) characterize the colonization of asymptomatic and symptomatic Monterey pine branches by beetle vectors and associated insects, (*ii*) determine insect and pathogen survival in chipped branches, and (*iii*) describe the survival of the pathogen in older branches and chips.

Methods

Branch collection

Four categories of Monterey pine branch tips were collected in February, May, August, and November of 1997 and 1998, at a coastal location adjacent to Spanish Bay Golf Course, Pebble Beach, Monterey County, California (36°37′N, 121°57′W), and at an inland site in Redwood Regional Park, Oakland, Alameda County, California (37°49′N, 122°10′W). Three disease-symptomatic branch categories exhibited characteristic resinosis and had either green, yellow, or red foliage. A control category consisted of branches that had green foliage and lacked visible symptoms of the disease. The Pebble Beach study site was within the Monterey peninsula native stand, whereas the Oakland study area was a planted site. Both sites were open, contained natural regeneration, and included all age-classes of Monterey pine. Sixty branch tips of each category were collected from each site during each collection period, and all branches were at least 2 m from other collected branches. Branch tips were 30–60 cm long and were collected from ground level up to approximately 6 m. Half were randomly selected to rear insects from intact branches and half were chipped using industrial chippers. To reduce the likelihood of cross-contamination, chipper blades and other accessible areas were sprayed with a 0.5% sodium hypochlorite solution before each branch category was chipped, and branch categories were chipped in order of increasing disease symptoms.

Insect incidence in branches and chips

To determine the species composition and phoresy rates of insects emerging from intact and chipped branches, branches and chips were incubated at the Insectary and Quarantine Facility, University of California, Berkeley, at 20 ± 2°C, ambient humidity, and under a 16-h photoperiod. Branches were incubated in black-plastic tubes (60 cm long × 10 cm diameter), and both ends were covered in dark cloth (Dallara 1997). At one end, a 2.5-cm transparent vial protruded through a cone-shaped sleeve to collect emerging insects. Chips were incubated in vertically oriented cardboard tubes with plastic coverings placed at both ends. The lower end contained a 1.2 cm diameter glass vial into which insects could walk, and which allowed light to shine into the tube. A 2.5 cm diameter hole in the side of the tube, covered with dark cloth, allowed air circulation. After 3 months of incubation, branches were individually packed in paper bags with a

plastic vial sealed around the open end and were incubated for an additional 3 months, to collect emerging *E. punctulatus*. Branches were measured to standardize insect emergence per unit length. Cardboard tubes were used only once, whereas all other materials were reused following either autoclaving or treatment with 0.5% sodium hypochlorite and rinsing.

Emerging insects were collected weekly, killed by freezing at –15°C, identified to genus (Drew 1963; Bright and Stark 1973; Wood 1982), partially embedded in *Fusarium*-selective medium in petri dishes using sterile technique (Correll *et al.* 1991), and incubated under an incandescent lamp at room temperature (range 19–22°C). At each collection, a random sample of approximately 5% of *Pityophthorus* spp. and 15% of *Lasconotus* spp. was subsequently removed from the medium for species identification. After 7–10 days, fungal colonies growing from insects were examined, and the identity of *F. circinatum* was confirmed by examination of the mycelial and microconidial structures (Nirenberg and O'Donnell 1998). Pathogenicity of *F. circinatum* isolates was tested on three occasions using the method of Correll *et al.* (1991), in which a total of 60 mycelium and agar samples were introduced into artificial wounds on potted Monterey pines. After 4 weeks, lesion lengths were compared with control wounds to which only sterile agar had been added. In all cases, pathogenicity of the tested isolate was confirmed.

Pathogen incidence in branches and chips

Incidence of the pathogen in fresh branches and branch chips was examined within several weeks of collection. Five pieces (1–2 cm × 1 cm × 2–3 mm) of bark, phloem, and xylem were removed from branches at locations where the pathogen was most likely to be present, such as adjacent to resin masses or at the margin of lesions. These pieces were then surface-treated with 0.5% sodium hypochlorite for 2 min, rinsed, and placed on *Fusarium*-selective medium using a sterile technique. The branch was returned to its plastic tube in one piece, and the remaining insects were allowed to complete development and emerge. Randomly selected chips from all branch and site categories were treated as described above. Three-month-old chips were combined into one tube for each combination of branch category and study site, and were incubated for an additional 3 months.

In March 1999, surface-treated samples of 1- and 3-year-old branches and 1-year-old chips were placed on selective medium, to determine whether the pathogen could be recovered from substrates stored under laboratory conditions for these periods. Branches were cut into 5- to 7-cm lengths, using sterile technique, and two pieces of each branch were placed on selective medium.

Statistical analysis

Hierarchical log-linear analysis was used to test for interactions between insect presence, phoresy, branch category, and site. *G* tests of independence were subsequently used to make orthogonal contrasts between branch categories and sites (Sokal and Rohlf 1981). Differences in the mean emergence per 30 cm of branch between consecutive branch categories at each site were tested using analysis of variance, and orthogonal contrasts were made between individual and pooled branch categories (Sokal and Rohlf 1981). Data were analyzed using SPSS for Windows 6.1 (SPSS Inc, Chicago).

Results

Insect incidence in branches and chips

The sample of twig beetles (n = 132) that emerged from Pebble Beach branches and were identified to species consisted of *Pityophthorus setosus* Blackman (38.4%), *P. carmeli* Swaine (36.4%), *P. nitidulus* (Mannerheim) (22.7%), and other *Pityophthorus* spp. (2.5%), whereas those emerging from Oakland branches (n = 519) were identified as *P. carmeli* (63.8%), *P. nitidulus* (32.9%), *P. setosus* (1.3%), and other *Pityophthorus* spp. (2.0%).

At both collection sites, less than 2% of asymptomatic and green symptomatic branches contained twig beetles during this study, whereas higher proportions of yellow and red branches were colonized. The mean ± SE proportions of yellow and red Pebble Beach branches from which at least one twig beetle emerged (54.2 ± 3.2 and 51.7 ± 3.2%, respectively) were not significantly different. At Oakland, twig beetles emerged from a greater proportion of red branches (63.8 ± 3.1%) than yellow branches (49.6 ± 3.2%) (G_1 = 9.81, P < 0.01). The mean number of emerged beetles per 30 cm of branch length varied significantly between collections in yellow and red branches at both sites, and no consistent patterns were observed (Fig. 1). At both Pebble Beach and Oakland, the mean number of emerged twig beetles varied by branch category (Table 1). Mean branch lengths varied between categories at both study sites (Pebble Beach: $F_{3,867}$ = 36.54, P < 0.001; Oakland: $F_{3,883}$ = 3.94, P = 0.008).

The overall phoresy rate of Pebble Beach twig beetles (23.0 ± 0.8%) was greater than the overall phoresy rate of Oakland twig beetles (5.5 ± 0.3%; G_1 = 590.24, P < 0.001). At both sites, there was considerable variation in twig beetle phoresy rates between collections (Fig. 2). At both study sites, the phoresy rate of twig beetles emerging from yellow branches was greater than the phoresy rate of twig beetles emerging from red branches (Table 1).

All *Lasconotus* spp. collected for species identification were *L. pertenuis* Casey, a result similar to Dallara (1997). At both sites, the mean ± SE proportions of yellow and red branches from which *Lasconotus* spp. emerged (Pebble Beach: 4.6 ± 1.4 *versus* 5.4 ± 1.5%; Oakland: 26.3 ± 2.9 *versus* 32.5 ± 3.0%) were not significantly different. The overall phoresy rate of *Lasconotus* spp. emerging from Pebble Beach branches (37.5 ± 7.8%) was greater than that from Oakland branches (16.1 ± 1.9%; G_1 = 9.19, P < 0.01). *Lasconotus* spp. emerged from 26.8 ± 1.9% of (combined) yellow and red branches from which *Pityophthorus* spp. had emerged, whereas 5.5 ± 1.1% of branches without *Pityophthorus* spp. emergence had at least one *Lasconotus* spp. emerge (G_1 = 83.56, P < 0.001).

At both sites, the mean ± SE proportions of yellow and red branches from which *Ernobius punctulatus* emerged (Pebble Beach: 6.3 ± 1.6 *versus* 7.9 ± 1.8%; Oakland: 5.8 ± 2.2 *versus* 7.5 ± 2.4%) were not significantly different. Overall phoresy rates of *E. punctulatus* emerging from Pebble Beach branches (6.7 ± 3.8%) and Oakland branches (0%) were not significantly different. Among all red and yellow branches, *E. punctulatus* emerged from 7.4 ± 1.1% of branches colonized by *Pityophthorus* spp. and from 3.0 ± 0.8% of branches without *Pityophthorus* spp. emergence (G_1 = 9.48, P < 0.02).

The numbers of other insects (Diptera, Hymenoptera, Homoptera, Hemiptera, other Coleoptera, *etc.*) and arthropods emerging from each combination of branch category and study site were similar (Table 1). The mean ± SE phoresy rate of these species was 5.5 ± 0.6% at Pebble Beach and 1.1 ± 0.3% at Oakland.

Nearly all twig beetles emerging from chipped branches emerged from the yellow or red categories (Table 2) and the total number of *Pityophthorus* spp. emerging from

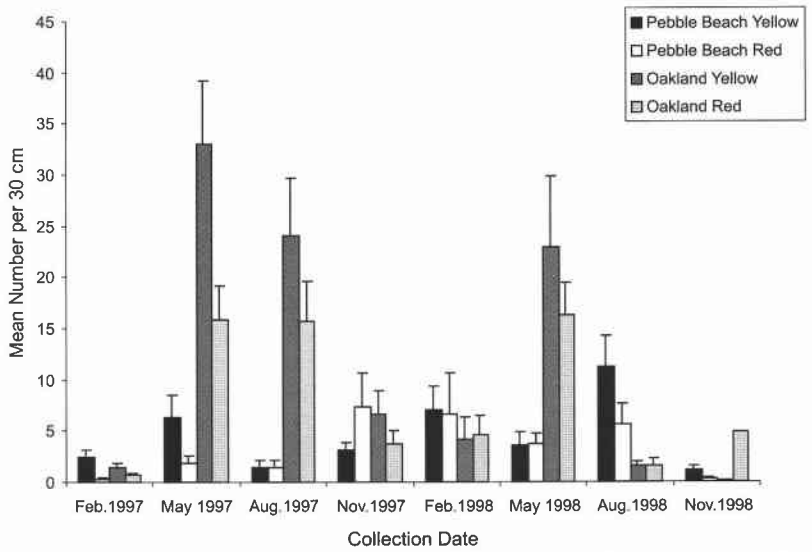

FIGURE 1. Mean + SE number of *Pityophthorus* spp. emerging from Pebble Beach and Oakland *Pinus radiata* branches at each collection date. Asymptomatic and green symptomatic branches are not shown.

chipped branches (n = 468) was lower than the number emerging from intact branches (n = 9872). Overall phoresy rates were higher among twig beetles emerging from chips than from branches (Pebble Beach: 48.4 ± 4.0 *versus* 23.0 ± 0.8%; Oakland: 16.3 ± 2.1 *versus* 5.5 ± 0.3%) (Pebble Beach: G_1 = 46.19, $P < 0.001$; Oakland: G_1 = 43.30, $P < 0.001$). Phoresy rates of twig beetles emerged from Pebble Beach branch chips varied between chip categories, whereas at Oakland, phoresy rates were independent of chip category (Table 2).

Pathogen incidence in branches and chips

The pathogen was isolated from Pebble Beach branches more frequently than from Oakland branches and, at both sites, the frequency of pathogen isolation varied between branch categories (Pebble Beach: G_3 = 243.19, $P < 0.001$; Oakland: G_3 = 77.72, $P < 0.001$). At Pebble Beach, the mean ± SE number of isolations per branch that yielded *F. circinatum* (maximum = 5) was asymptomatic, 0.22 ± 0.04; green symptomatic, 0.58 ± 0.07; yellow, 1.48 ± 0.10; and red, 1.90 ± 0.10. In Oakland branches, the mean number of isolations was asymptomatic, 0.07 ± 0.02; green symptomatic, 0.09 ± 0.03; yellow, 0.38 ± 0.06; and red, 0.58 ± 0.07.

Isolation frequencies from yellow and red branches sampled soon after collection and after 1 year of incubation were not significantly different at either site. The pathogen was isolated from 68.4 ± 7.6% of 1-year-old Pebble Beach branches (n = 38) and from 21.1 ± 6.7% of 1-year-old Oakland branches (n = 40). In a sample of 3-year-old Pebble Beach branches, the pathogen was recovered from only 1 of 46 yellow and red branches sampled. Of branches sampled twice (when recently collected and after 3 years of incubation), isolation frequencies were lower after 3 years (0%) than when recently collected (73.3 ± 8.1%; G_1 = 39.20, $P < 0.001$).

At both Pebble Beach and Oakland, temporal isolation frequencies from branch chips were highly variable. The frequency of pathogen isolation from branch chips increased with an increasing severity of disease symptoms and varied significantly

TABLE 1. Mean ± SE emergence per 30 cm of branch length and phoresy rates (%) of pitch canker-associated taxa from *Pinus radiata* branches.

	Pityophthorus spp.		*Lasconotus* spp.		*Ernobius punctulatus*		Other arthropods	
Branch category	No. per 30 cm	Phoresy rate (%)	No. per 30 cm	Phoresy rate (%)	No. per 30 cm	Phoresy rate (%)	No. per 30 cm	Phoresy rate (%)
				Pebble Beach				
Asymptomatic	0.01±0.01*a*	0	0.01±0.00*a*	100	0.01±0.01*a*	0	0.84±0.09*a*	2.9±1.0*a*
Green symptomatic	0.01±0.01*a*	11.1±11.1*a*	0.0±0.0*a*	0	0.01±0.01*a*	0	0.86±0.11*a*	1.6±0.6*a*
Yellow symptomatic	4.3±0.6*b*	26.7±1.3*a*	0.05±0.02*a*	43.8±13.2*a*	0.05±0.01*a*	13.3±9.4*a*	0.90±0.11*a*	12.5±1.9*b*
Red symptomatic	3.8±0.7*b*	20.5±1.0*b*	0.06±0.03*a*	28.6±10.4*a*	0.08±0.02*a*	4.4±4.4*a*	0.94±0.10*a*	6.0±1.3*c*
				Oakland				
Asymptomatic	0.01±0.01*a*	0	0.0±0.0*a*	0	0.01±0.01*a*	0	0.85±0.11*a*	0.4±0.4*a*
Green symptomatic	0.2±02*a*	0*a*	0.03±0.02*a*	11.1±11.1*a*	0.02±0.01*a*	0	1.20±0.17*a*	0*a*
Yellow symptomatic	12.1±1.7*b*	6.9±0.4*b*	0.65±0.11*b*	16.8±2.7*a*	0.03±0.01*a*	0	0.95±0.12*a*	1.3±0.7*b*
Red symptomatic	6.9±0.9*c*	3.6±0.4*c*	0.49±0.08*b*	15.6±2.7*a*	0.05±0.02*a*	0	0.78±0.11*a*	3.1±1.0*b*

NOTE: Significant differences between consecutive branch categories at each study site are indicated by different letters (G test, $P < 0.05$). No letter indicates that a comparison was not made, owing to small sample size.

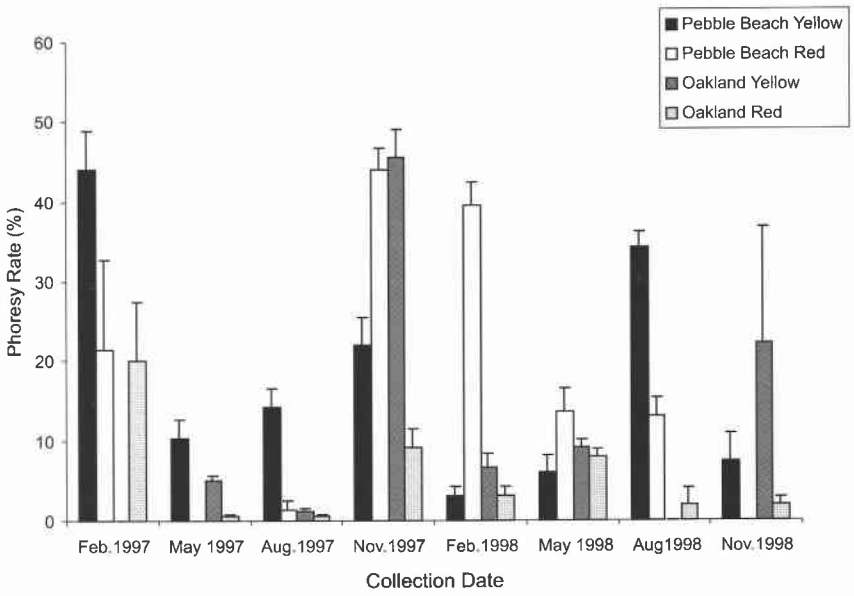

FIGURE 2. Mean + SE phoresy rates of *Pityophthorus* spp. emerging from Pebble Beach and Oakland *Pinus radiata* branches at each collection date. Asymptomatic and green symptomatic branches are not shown.

TABLE 2. Number (n) and mean ± SE phoresy rate (%) of pitch canker associated taxa emerging from chipped *Pinus radiata* branches incubated under laboratory conditions.

	Pityophthorus spp.		*Lasconotus* spp.		Other arthropods	
Chip category	n	Phoresy rate (%)	n	Phoresy rate (%)	n	Phoresy rate (%)
			Pebble Beach			
Asymptomatic	0	0	0	0	0	0
Green symptomatic	0	0	0	0	0	0
Yellow symptomatic	83	31.3±5.1*a*	0	0	1	0
Red symptomatic	78	66.7±5.4*b*	2	50.0±50.0	0	0
			Oakland			
Asymptomatic	1	0	0	0	1	0
Green symptomatic	0	0	0	0	1	0
Yellow symptomatic	239	16.3±2.4*a*	6	0	15	0
Red symptomatic	67	16.4±4.6*a*	6	33.3±21.1	2	50.0±50.0

NOTE: Significant differences between consecutive chip categories at each study site are indicated by different letters (G test, $P < 0.05$). No letter indicates that a comparison was not made, owing to small sample size. No *Ernobius punctulatus* emerged from chipped branches.

between chip categories (Table 3). Pathogen isolation was more frequent from red chipped branches than from yellow chipped branches.

Isolation frequencies from 1-year-old yellow and red Pebble Beach branch chips were lower than in chips from the same collection that were plated soon after chipping (yellow: $G_1 = 96.66$, $P < 0.001$; red: $G_1 = 53.57$, $P < 0.001$), but isolation frequencies from 1-year-old and freshly plated Oakland branch chips were not significantly different (Fig. 3).

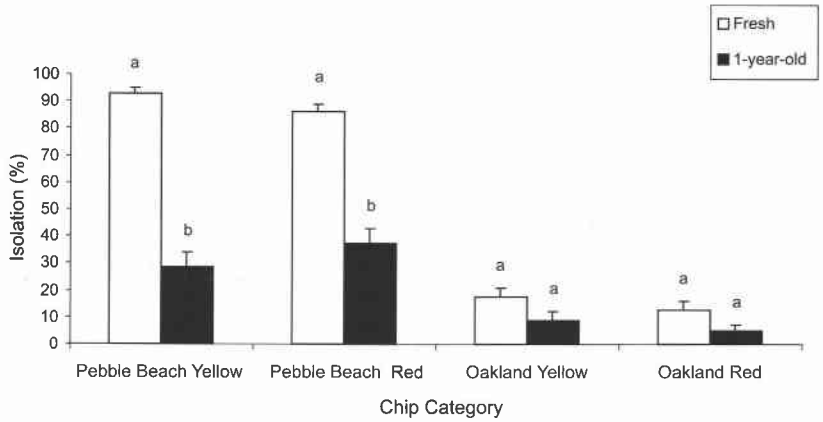

FIGURE 3. Mean + SE frequency of *Fusarium circinatum* isolation from freshly plated and 1-year-old *Pinus radiata* chips. For each category, significant differences between ages are indicated by different letters (*G* test, $P < 0.05$).

TABLE 3. Mean ± SE frequency of *Fusarium circinatum* isolation from surface-treated chipped *Pinus radiata* branches.

Chip category	*n*	Isolation (%)	Range (%)
		Pebble Beach	
Asymptomatic	866	46.5±1.7*a*	20.0±3.4 – 89.1±2.9
Green symptomatic	929	48.2±1.7*a*	23.4±3.8 – 91.2±2.5
Yellow symptomatic	881	63.7±1.6*b*	31.2±4.0 – 92.9±2.3
Red symptomatic	901	73.6±1.5*c*	59.8±4.6 – 100.0
		Oakland	
Asymptomatic	987	15.6±1.2*a*	0.9±0.9 – 30.9±4.2
Green symptomatic	1004	16.7±1.2*a*	3.4±1.7 – 55.1±5.1
Yellow symptomatic	1052	28.2±1.7*b*	2.9±1.6 – 96.1±1.7
Red symptomatic	1066	36.1±1.5*c*	10.8±2.9 – 97.8±1.3

NOTE: Significant differences between consecutive chip categories at each study site are indicated by different letters (*G* test, $P < 0.05$).

Discussion

In the absence of pitch canker, *Pityophthorus* spp. attack weakened or recently killed branches (Furniss and Carolin 1977). In this study, symptomatic branches with green foliage were seldom colonized by *Pityophthorus* spp., in contrast to frequent twig beetle colonization in symptomatic branches with discolored foliage. It is important to note that all symptomatic branch categories yielded insects carrying the pathogen. The higher mean emergence from yellow branches than from red branches at both sites is likely the result of yellow branches being at an earlier stage of colonization. Yellow branches that did not contain beetles may have been too vigorous for colonization or were suitable for colonization but had not yet been located by twig beetles at the time of collection. Many susceptible branches were not colonized, likely owing to low beetle landing rates (Moeck *et al.* 1981; Bonello *et al.* 2001) and the inability of twig beetles to detect weakened or cut branches prior to landing on them (Bonello *et al.* 2001). Twig beetles had completed their development and emerged from a portion of branches with

red foliage, whereas other branches with red foliage had no signs of twig beetle colonization when dissected.

At both sites, the presence of *Pityophthorus* spp. in branches varied between branch categories, months of collection, and years. At Pebble Beach, moderate year-round temperatures may account for the lack of consistent emergence patterns. Farther inland at Oakland, a greater proportion of branches contained twig beetles during the May and August collections. This may be due to greater water stress further weakening diseased branches (Schmidt *et al.* 1976). In addition, warmer inland temperatures may permit a longer period of beetle flight, thus resulting in a greater likelihood that susceptible branches will be located and colonized.

Lasconotus spp. were seldom obtained from branches not colonized by twig beetles, consistent with the fact that *Lasconotus* spp. are known predators of bark beetles (Hackwell 1973; Furniss and Carolin 1977). Many branches in the early stage of colonization by twig beetles may not yet have been discovered by *Lasconotus* spp. at the time of collection (Rohlfs and Hyche 1981, 1984). A larger twig beetle population at the Oakland study site is likely responsible for the greater *Lasconotus* spp. emergence than at Pebble Beach. It is believed that phoresy rates of *Lasconotus* spp. were higher than those of *Pityophthorus* spp., owing to movement beneath the bark while searching twig beetle galleries for prey (Dallara 1997). More *E. punctulatus* emerged from yellow and red branches than from branches with green foliage, as expected, because these beetles preferentially colonize dead branches (Furniss and Carolin 1977). *Ernobius punctulatus* has also been reported to be commonly present on the foliage of healthy trees (Ohmart 1981). The absence of phoretic *E. punctulatus* in Oakland reflected the lower incidence of the pathogen in diseased branches. Other insects and arthropods were collected in similar numbers in all branch categories at both sites, but some species, such as wasps and flies, were associated with branches that were in a later stage of twig beetle colonization. Phoresy rates reflected the progression of disease symptoms, in that insects emerging from yellow and red branches had higher phoresy rates than those emerging from branches with green foliage.

The pathogen was isolated from Pebble Beach branches with greater frequency than from Oakland branches. Pathogen survival in host branches may be higher in cooler coastal locations than at inland sites. In addition, higher phoresy rates were found for beetles that emerged from Pebble Beach branches. At both sites, the pathogen was isolated from branches and chips with increasing frequency as disease symptoms increased. Yellow and red branches may have been inoculated more than once, and the pathogen was more likely to have spread through the branch prior to collection than in green symptomatic branches. Many resinous lesions and resin beads in green symptomatic branches may have been due to causes other than pitch canker.

The number of *Pityophthorus* spp., *Lasconotus* spp., *E. punctulatus*, and other species emerged from chips, compared with the number emerging from branches, indicated that chipping reduced the emergence of insects present in infested branches by approximately 95% under laboratory conditions. On average, one or two phoretic twig beetles emerged from each yellow or red branch, whereas one phoretic twig beetle emerged from 5–20 chipped branches. It is possible that some of this effect was due to different containers affecting the rates of branch and chip desiccation. Phoresy rates were higher for insects emerging from chips than for those emerging from intact branches. This may be a result of the orientation of the chips during incubation, in that beetles may acquire inoculum while walking on a greater surface area of contaminated chips than on intact branches. High pathogen isolation rates from asymptomatic chipped branches may reflect a difficulty in completely sterilizing the chipper or imperfect surface treatment following chipping.

This study demonstrates that *F. circinatum* is capable of surviving in pine branches from which all insects are known to have emerged. It is also possible that spores or other inoculum may enter the soil of a disease-free area where recently cut branches or chips have been placed (Dwinell and Barrows 1978). At both study sites, the pathogen was frequently isolated from 1-year-old branches and chips, whereas in 3-year-old Pebble Beach branches, the pathogen was recovered only once. There is a significant risk of spreading pitch canker to uninfested areas through the movement of recently cut branches and chips, but the risk should diminish as the branches and chips age. Therefore, it is recommended that recent branch cuttings and chips originating from symptomatic trees not be transported to areas that are believed to be free of the disease.

Acknowledgements

We thank the Pebble Beach Company and the East Bay Regional Parks District for use of the two study sites, and the Pebble Beach Company Forestry Division and the California Department of Transportation, Telegraph Avenue Maintenance Station for their assistance in chipping branches. Funding for this study was provided by the Pebble Beach Company and the California Department of Transportation. We thank Pierluigi Bonello and two anonymous referees for their reviews of the manuscript.

References

Bonello P, McNee WR, Storer AJ, Wood DL, Gordon TR. 2001. The role of olfactory stimuli in the location of weakened hosts by twig infesting *Pityophthorus* spp. (Coleoptera: Scolytidae). *Ecological Entomology* **26**: 8–15

Bright DE Jr, Stark RW. 1973. The bark and ambrosia beetles of California (Coleoptera: Scolytidae and Platypodidae). *Bulletin of the California Insect Survey* **16**

Correll JC, Gordon TR, McCain AH, Fox JW, Koehler CS, Wood DL, Schultz ME. 1991. Pitch canker in California: pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). *Plant Disease* **75**: 676–82

Dallara PL. 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. PhD dissertation, University of California, Berkeley

Dallara PL, Storer AJ, Gordon TR, Wood DL. 1995. Current status of pitch canker disease in California. *California Department of Forestry and Fire Protection Tree Note* **20** [California Department of Forestry and Fire Proteciton, 1416 Ninth Street, Sacramento, California, United States 95814]

Drew JK. 1963. A revision of the genus *Lasconotus* Erichson in California (Coleoptera, Scolytidae). MS thesis, University of California, Berkeley

Dwinell LD, Barrows JB. 1978. Recovery of the pine pitch canker fungus (*Fusarium moniliforme subglutinans*) from pine (*Pinus taeda*) plantation and seed orchard soil. *Phytopathology News* **12**: 207 [American Phytopathological Society, 3340 Pilot Knob Road, St. Paul, Minnesota, United States 55121]

Dwinell LD, Barrows-Broaddus JB, Kuhlman EG. 1985. Pitch canker: a disease complex of southern pines. *Plant Disease* **69**: 270–6

Fox JW, Wood DL, Koehler CS. 1990. Distribution and abundance of engraver beetles (Scolytidae: *Ips* species) on Monterey pines infected with pitch canker. *The Canadian Entomologist* **122**: 1157–66

Fox JW, Wood DL, Koehler CS, O'Keefe ST. 1991. Engraver beetles (Scolytidae: *Ips* species) as vectors of the pitch canker fungus, *Fusarium subglutinans*. *The Canadian Entomologist* **123**: 1355–67

Furniss RL, Carolin VM. 1977. Western forest insects. *United States Department of Agriculture Forest Service Miscellaneous Publication* **1339**

Gordon TR, Okamoto D, Storer AJ, Wood DL. 1998*a*. Susceptibility of five landscape pines to pitch canker disease, caused by *Fusarium subglutinans* f.sp. *pini*. *Hortscience* **33**: 868–71

Gordon TR, Wikler KR, Clark SL, Okamoto D, Storer AJ, Bonello P. 1998*b*. Resistance to pitch canker disease, caused by *Fusarium subglutinans* f.sp. *pini*, in Monterey pine (*Pinus radiata*). *Plant Pathology* **47**: 706–11

Hackwell GA. 1973. Biology of *Lasconotus subcostulatus* (Coleoptera: Colydiidae) with special reference to feeding behavior. *Annals of the Entomological Society of America* **66**: 62–5

Hoover K, Wood DL, Storer AJ, Fox JW, Bros WE. 1996. Transmission of the pitch canker fungus, *Fusarium subglutians* f.sp. *pini*, to Monterey pine, *Pinus radiata*, by cone- and twig-infesting beetles. *The Canadian Entomologist* **128**: 981–94

McCain AH, Koehler CS, Tjosvold SA. 1987. Pitch canker threatens California pines. *California Agriculture* **41**: 22–3

Moeck HA, Wood DL, Lindahl KQ. 1981. Host selection behavior of bark beetles (Coleoptera: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*. *Journal of Chemical Ecology* **7**(1): 49–83

Nirenberg HI, O'Donnell K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* **90**: 434–58

Ohmart CP. 1981. An annotated list of insects associated with *Pinus radiata* D. Don in California. *CSIRO Division of Forest Research Report* **8**

Rohlfs WM III, Hyche LL. 1981. Colydiidae associated with *Ips* in southern pines: relative abundance and time of arrival of adults at pines under attack by *Ips* spp. *Journal of Economic Entomology* **74**: 458–60

——— 1984. Observations on activity and development of *Lasconotus pusillus* and *L. referendarius* (Coleoptera: Colydiidae) following arrival at *Ips* spp.-infested southern pines. *Journal of the Georgia Entomological Society* **19**: 114–9

Schmidt RA, Wilkinson RC, Moses CS, Broerman FS. 1976. Drought and weevils associated with severe incidence of pitch canker in Volusia County, Florida. *University of Florida Institute of Food and Agricultural Sciences Program Report* **76-2**

Sokal RR, Rohlf FJ. 1981. *Biometry*. 2nd edition. New York: WH Freeman and Co

Storer AJ, Dallara PL, Wood DL, Gordon TR. 1994. Pitch canker in California: geographic and host range expansion. *California Agriculture* **48**(6): 9–13

Storer AJ, Gordon TR, Wood DL, Bonello P. 1997. Pitch canker disease of pines: current and future impacts. *Journal of Forestry* **95**(12): 21–6

Storer AJ, Wood DL, Wikler KR, Gordon TR. 1998. Association between a native spittlebug (Homoptera: Cercopidae) on Monterey pine and an introduced tree pathogen which causes pitch canker disease. *The Canadian Entomologist* **130**: 783–92

Storer AJ, Wood DL, Gordon TR. 1999. Modification of co-evolved insect–plant interactions by an exotic plant pathogen. *Ecological Entomology* **24**: 238–43

Wood SL. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Naturalist Memoirs* **6**

(Received: 2 August 2001; accepted: 17 October 2001)