

Pitch Canker Disease in California: Pathogenicity, Distribution, and Canker Development on Monterey Pine (*Pinus radiata*)

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ABSTRACT

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Pitch canker disease, caused by *Fusarium subglutinans*, has become prevalent on Monterey pine (*Pinus radiata*) in several central and southern coastal counties of California. Although Monterey pine is the most frequently infected host, the pathogen has also been recovered from bishop (*P. muricata*), Aleppo (*P. halepensis*), and Canary Island (*P. canariensis*) pine. All 167 isolates of *F. subglutinans* recovered from diseased tissue, insects, and air samples in California were virulent on Monterey pine in greenhouse pathogenicity tests, and 63 pine isolates of *F. subglutinans* from Florida, North Carolina, and Texas were also virulent on Monterey pine. All isolates of *F. subglutinans* from plant hosts other than pine, as well as pine and nonpine isolates of *F. proliferatum* and *F. moniliforme*, were avirulent or weakly virulent on Monterey pine and considered nonpathogenic. Airborne inoculum of *F. subglutinans* was detected throughout the year in Santa Cruz County in an area with a high incidence of pitch canker disease but was not detected in an area where the disease was absent. *F. subglutinans* was also recovered from numerous insect species in this area, many of which are capable of feeding on Monterey pine and causing wounds. Inoculation data in this study provide considerable justification for assigning strains of *F. subglutinans* pathogenic to pines to a specific formae specialis. We therefore propose that the pitch canker pathogen be designated *F. subglutinans* f. sp. *pini*. Isolations and field inoculations indicated that different aged branch tissue, cones, and boles were susceptible infection courts throughout the year. Extensive resin-soaked cankers developed from branch and bole inoculations in less than 2 yr. The significant differences in canker development observed among field-inoculated Monterey pine trees may reflect genetic variation in susceptibility to pitch canker disease within the population of planted Monterey pines in California.

Keywords: epidemiology, *Fusarium moniliforme* var. *subglutinans*, *Fusarium* section *Liseola*

Pitch canker disease of pines, caused by *Fusarium subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussein, & Marasas (*F. moniliforme* J. Sheld. var. *subglutinans* Wollenweb. & Reinking), was first reported in North Carolina in 1946 (20). The disease is considered to be endemic in the southeastern United States and occurs north to Virginia, south to Florida, and west to Texas (14). Losses from tree mortality, reduced lumber quality because of stem deformation, reduced growth, seed contamination in seed orchards, and seedling mortality in nurseries have been extensive (1,3,14,31). In Florida, pitch canker was the primary cause of tree mortality in a slash pine (*Pinus elliotii* Engelm. var. *elliotii*) plantation where other biotic and abiotic factors affecting trees were evaluated (7).

Several studies in the southeastern United States have examined the epidem-

iology of pitch canker (1,3-6,14,23-26, 31,32). Airborne inoculum and several insect vectors have been implicated as important means of spreading the pathogen. The deoder weevil (or eastern pine weevil), *Pissodes nemorensis* Germar, in particular, has been demonstrated to be an important vector of the pitch canker pathogen in Florida (8). However, other insects, acting both as vectors and as wounding agents, apparently contribute to disease spread (14,27, 31,32). Other factors, such as differential species and/or clone susceptibility, high soil fertility, and water stress, also have been shown to increase disease severity (10,15,18,30,32). Genetic resistance (tolerance) to pitch canker has been demonstrated within populations of slash and loblolly pine (*P. taeda* L.) (25,30). Although pitch canker continues to be a production constraint in the southeastern United States, effective management procedures and the use of resistant host material have helped to reduce its overall impact (14,30).

Pitch canker was first identified in Santa Cruz County, California, on Monterey pine (*P. radiata* D. Don) in the summer of 1986 (28). However,

Hepting (19) had reported as early as 1961 that *P. radiata* was susceptible to pitch canker. The disease has been observed on Monterey pine planted predominantly along roadway right-of-ways and in landscape settings in central coastal areas of California and has become quite severe in some locations. At present, very little is known about the epidemiology of this disease in California.

The purpose of this study was to determine if isolates of *F. subglutinans* recovered from diseased pine tissue, contaminated insects, and air samples collected throughout California where the disease was found were virulent on Monterey pine; air samples were also collected in areas where pitch canker was absent. In addition, the extent of canker development on Monterey pine in field inoculation experiments under California environmental conditions was determined.

MATERIALS AND METHODS

Isolations. A modified Nash-Snyder medium (29), designated FS medium, was used for all plant tissue, insect, and air isolations. FS medium consisted of 15 g of peptone, 1 g of KH_2PO_4 , 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g of pentachloronitrobenzene (PCNB 75%), 20 g of agar, and 1 L of H_2O . After the medium was autoclaved and allowed to cool to approximately 60 C, 0.075 g of chloroneb (65%), 0.05 g of triadimefon (25%), 0.1 g of ampicillin, and 0.02 g of rifampicin were added.

The distribution of the pathogen was determined by surveying locations with relatively mature Monterey pine stands in California between 1987 and 1990. The survey typically included a ground survey of trees along a freeway corridor. Many individual tree locations were brought to our attention by local farm advisors or homeowners.

Isolations were made from young branch tips, 1- to 10-yr-old branches, boles, and cones of Monterey pine with symptoms of pitch canker. Isolations were also made from symptomatic tissue collected from several other pine species throughout the state, including bishop (*P. muricata* D. Don), Aleppo (*P. halepensis* Mill.), and Canary Island (*P. canariensis* Chr. Sweet ex Spreng.) pine. Symptoms of pitch canker usually

included oozing resin near the canker and an amber-colored, resin-soaked appearance of the tissue beneath the bark. Pieces of amber, resin-soaked wood were cut from the canker margin, soaked in 30% commercial bleach (1.6% sodium hypochlorite) for 1 min, rinsed with sterile water, and plated on FS medium. Isolation plates were incubated at room temperature (23 ± 4 C) under a 12-hr light/12-hr dark cycle. Isolates of *Fusarium* in section *Liseola* were cultured on carnation leaf agar (CLA) amended with 6.0 g/L of KCl for species identification (16). Cultures for pathogenicity tests were grown on potato-dextrose agar (PDA) at 25 ± 1 C under a 12-hr light/12-hr dark cycle for 7–10 days. Isolations from samples in Florida were made from branch tips and branches taken primarily from symptomatic tissue from the tops of slash pine trees. All Florida isolates were recovered in May 1988.

Pine and nonpine isolates of *F. subglutinans*, *F. proliferatum* (T. Matsushima) Nirenberg, and *F. moniliforme* were also obtained from various researchers. Forty-four isolates from diverse hosts and geographic locations were also tested for pathogenicity on Monterey pine.

All isolates except those recovered from air sample plates were single-spored by means of a stage-mounted micro-manipulator. Isolates recovered from air sample plates were mass-transferred to PDA plates.

Insect collections. Insects were collected on sticky traps placed adjacent to diseased trees or while in free flight in multiple-funnel traps (23) placed within 50 m of infected trees in Santa Cruz County. Sticky traps were constructed of 25×50 cm plastic screens coated with Tangle Trap (Tanglefoot Co., Grand Rapids, MI). Insects were frozen and placed on FS medium, and all fungal colonies emerging from insects were subcultured back to FS medium.

Air samples. Petri plates (9.5 cm diameter) containing 25 ml of FS medium were used to sample the air for the pitch canker pathogen. Air samples were taken in two areas. The first sample area in Santa Cruz County was along a 24-km north-south corridor of California Highway 1 and Highway 17, from Seacliff State Beach north to Scotts Valley, where over 1,000 Monterey pine trees had visible symptoms of pitch canker. Seven of the 12 sample sites were immediately adjacent to Highway 1, two were on cliffs overlooking the ocean approximately 0.5 km from the freeway, and three were within 50 m of the freeway. The second sample area was farther north on Highway 1 in a native stand of Monterey pine near Ano Nuevo State Park. Although pitch canker had never been observed in this stand of Monterey pine, the pathogen was recovered from a single branch from one tree planted adjacent to the highway.

Air sample plates were placed horizontally, with the medium facing upward, on top of 0.5-m-tall guardrail posts adjacent to the freeway. On each sample date, three to six plates were placed approximately 10 m apart at each site. Plates were deployed at 1800–2000 and recovered at 0600–0800 for a 12-hr exposure period.

Greenhouse pathogenicity tests. Greenhouse pathogenicity tests were performed on 2- to 3-yr-old Monterey pine seedlings grown in potting mix (peat/sand) and fertilized one to three times a week with dilute Hoagland's solution (25). Greenhouse temperatures fluctuated daily but were approximately 27 ± 6 C. Each isolate tested was grown on PDA for 7–10 days as previously described. Inoculum (mycelium and microconidia) was scraped off the agar surface with a sterile transfer needle, which was then used to make a 0.5-cm-long slit wound into the succulent branch tissue and thereby introduce the inoculum. The wound was made parallel to the axis of the branch and between 5 and 10 cm behind the growing point of the inoculated branch. Each inoculation was replicated three times, with each replication on a different seedling. All pathogenicity tests were repeated at least once.

Inoculated seedlings were scored for disease symptoms after 4, 6, and 8 wk. Each inoculation was rated on a scale of 0–4, where 0 = healthy, no necrosis; 1 = healthy foliage, necrosis only at the point of inoculation; 2 = healthy foliage, necrosis > 2 cm beyond the point of inoculation; 3 = needles and/or branch wilting and necrosis girdling branch; and 4 = branch girdled and foliage dead distal to the point of inoculation.

Field experiments. Experiment A was conducted in Santa Cruz County on mature Monterey pine trees that had different levels of disease before the experiment. Disease severity was estimated on all trees before and after each experiment. Tree age was determined by taking an increment core sample from each bole at 1.4 m above the ground. The experiment was designed to determine the rate and extent of canker development, the susceptibility of different infection courts, and the effect of time of year of inoculation on infection.

The plant parts challenged with the pathogen were branch tips (< 2 cm in diameter), branches (5–10 cm in diameter), the distal end of immature cones (< 6 cm long, immature green tissue), the distal end of mature cones (> 8 cm long, mature brown tissue), and boles (main trunk). Infection courts were prepared by making a wound through the bark and into the cambium with a sterilized nail (0.3 cm diameter); for bole inoculations, a larger nail (0.5 cm diameter) was used. For both mature and immature cones, a 3- to 6-mm-deep

wound was made into the distal end of each cone.

Isolate FK863 of *F. subglutinans* was used for all inoculations. This isolate was recovered originally from a diseased Monterey pine in Santa Cruz County in 1987 and was virulent in greenhouse pathogenicity tests. This isolate was grown on PDA, and inoculum was prepared by washing conidia off the agar surface with sterile water and adjusting the spore concentration to 1×10^6 spores per milliliter. Approximately 0.1 ml of the spore suspension was placed in each branch and cone wound and 0.5 ml in each bole wound. Controls consisted of a similar wound on all tissue followed by inoculation with sterile water.

Trees were inoculated in November 1987 and March and July 1988. Each infection court was inoculated at two locations on each tree and was replicated on five separate trees. Data were analyzed as a randomized complete block design in which each tree was a block.

Branch tip and cone inoculations were scored every 4–8 wk after inoculation. Branch tips were rated on a scale of 0–5, where 0 = healthy; 1 = dry wound, no foliar symptoms; 2 = resinosis at wound, no foliar symptoms; 3 = resinosis at wound and/or sunken lesion with foliar chlorosis; 4 = resinosis at wound and chlorotic/necrotic needles distal to the point of inoculation; and 5 = girdling lesion with most ($> 75\%$) of the needles distal to the inoculation point necrotic. Cones were rated as either positive or negative for visible development of an infection. All branch inoculations were harvested in June 1989 and returned to the laboratory. The bark was carefully removed from around the inoculation point, and the length and width of each exposed canker were measured. In June 1989, bole cankers were measured, in situ, by paring away the bark.

Experiment B was also conducted in Santa Cruz County, approximately 8 km south of experiment A on similar aged trees. At the second location, branch tips (< 2 cm diameter) and two size classes of branches, 3–5 and 5–8 cm diameter, were inoculated. At the start of the experiment, three of the six trees (trees 2, 4, and 6) inoculated had numerous pitch canker infections (40–85% of branches infected), whereas the other three (trees 1, 3, and 5) had very few (0–5%) infections. The three trees with fewer natural field infections were designated as putatively resistant. The experiment was designed as a randomized complete block where, again, each tree served as a block. Five separate locations were inoculated per treatment per tree.

Branches were inoculated as described previously except that five small wounds (0.2–0.3 cm diameter) were made on each branch within a 2×2 cm area. A 0.05-ml drop of inoculum, prepared as previously described, was placed in each

Table 1. Recovery of *Fusarium subglutinans* from air sample plates in Santa Cruz County^a

Site	Sample date													
	1987					1988								
	11/4	1/3	2/4	3/2	3/15	4/13	4/15	5/5	5/18	7/7	7/20	8/18	9/8	10/6
Emeline north	0.7 ^b	0.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Emeline south	0.4	1.0	0.0	0.3	0.0	1.3	0.0	1.0	0.3	1.0	0.7	0.3	0.0	0.3
Emeline entrance	... ^c	2.0	52.7	1.0	1.5	0.0	0.5	0.2	0.7	0.2	9.8	0.7	0.0	0.0
Emeline Highway	0.8	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0
New Brighton kiosk	0.3	0.3	0.3	0.3	...	2.3	0.0	7.7	0.3	...	0.0	0.0	0.0	0.0
New Brighton cliff	1.0	1.3	11.7	1.5	13.8	0.0	1.0	7.7	0.5	0.0	0.3
New Brighton Highway	0.0	0.0	1.0	0.0	7.0	0.0	0.3	0.0	0.0	0.3	0.0
Bay street	...	0.3	3.0	2.2	2.3	0.0	0.7	4.7	2.0	0.7	0.5	0.0	0.3	0.2
Pasatiempo site	0.0	0.0	0.0	0.0	...	2.0	1.3	0.3	0.0	0.0	0.0
Seacliff cliff	0.0	0.0	0.3	0.0	11.0	0.0	0.0	2.0	0.0	0.3	0.0
Seacliff Highway	0.0	...	0.3	0.3	0.0	0.0	0.0	0.7	0.0	0.0	0.0
Madrona site	0.3	...	0.0	...	0.7	0.0	0.3	0.0	0.0	0.0

^a Plates were put out at 1800–2000 and recovered at 0600–0800 the following morning. No colonies of *F. subglutinans* were recovered from air sample plates from nine sites near the Ano Nuevo State Park, where no pitch canker was present.

^b Each number is the mean of colonies of *F. subglutinans* recovered from three to six isolation plates. A subsample of colonies indicated that all colonies with a characteristic morphology were pathogenic.

^c Sample not taken.

wound. All these trees were inoculated in May 1988. Branch tip inoculations were scored every 4–8 wk. Branch inoculations were harvested in June 1989 and returned to the laboratory, where the bark was removed to measure the extent of canker development.

RESULTS

Pathogen morphology. On CLA amended with 6.0% KCl, isolates of *F. subglutinans* pathogenic to pine produced abundant microconidia exclusively in false heads. Polyphialides producing microconidia also were readily observed on this medium after 1 wk. The extent of macroconidial production on CLA was quite variable among the pathogenic isolates. Although no microscopic or morphological differences between the pathogenic and nonpathogenic isolates of *F. subglutinans* were observed, there were quantitative differences in microconidia and macroconidia production.

All rice isolates of *F. subglutinans* produced microconidia in false heads, but an occasional “chain” of microconidia was found in which the microconidia appeared to be stuck together side-to-side rather than end-to-end in true chains. All pine and nonpine isolates of *F. moniliforme* and *F. proliferatum* produced microconidia in true chains. In general, isolates of *F. proliferatum* produced shorter chains of microconidia than did isolates of *F. moniliforme*. Polyphialides were evident in all cultures of *F. proliferatum* but sometimes were difficult to find.

Air sampling. Recovery of *F. subglutinans* on air sample plates varied according to both the site and the sample date. *F. subglutinans* was recovered on air sample plates from 25–100% of the sample sites for all sample dates throughout the year from an area in Santa Cruz County with a very high incidence of pitch canker (Table 1). The mean number of colonies of *F. subglutinans* recovered

on isolation plates also varied (Table 1). *F. subglutinans* was not recovered from 66 of the 136 samples, and 49 samples had 1.0 or fewer colonies per plate. Four samples had > 10 colonies per plate, with the highest count at 52.7 colonies per plate (Table 1). Several *Fusarium* spp. other than *F. subglutinans* were recovered on air sample plates, including *F. oxysporum* Schlechtend.:Fr., *F. solani* (Mart.) Sacc., *F. equiseti* (Corda) Sacc., and *F. proliferatum*. *F. subglutinans* was not recovered at any of nine sites near Ano Nuevo State Park (where pitch canker did not occur) sampled on 20 July, 18 August, and 8 September 1988 (data not shown).

Pathogenicity tests. In greenhouse pathogenicity tests, an isolate producing a mean disease rating of ≥ 3.5 at 6 wk after inoculation was considered pathogenic on Monterey pine, whereas an isolate producing a mean disease rating of ≤ 1.5 at 6 wk was considered nonpathogenic. A disease rating of 1.5 indicated that some localized necrosis but no foliar symptoms typical of the pathogen had occurred at the point of inoculation.

Ninety-two isolates of *F. subglutinans* recovered from pine with symptoms of pitch canker from over 20 locations in eight counties of California (Fig. 1) were tested in the greenhouse for pathogenicity on Monterey pine (Table 2). All pine isolates of *F. subglutinans* from California (81 from Monterey, 6 from bishop, 4 from Aleppo, and 1 from Canary Island pine) were virulent in greenhouse pathogenicity tests. In addition, all 36 insect isolates and all 39 air sample isolates of *F. subglutinans* were virulent. The pine-feeding insect species from which a pathogenic isolate of *F. subglutinans* was recovered included the dry cone and twig beetle (*Ernobius punctulatus* Le Cont), the Monterey pine and California firespined engraver beetles (*Ips mexicanus* Hopkins and *I.*

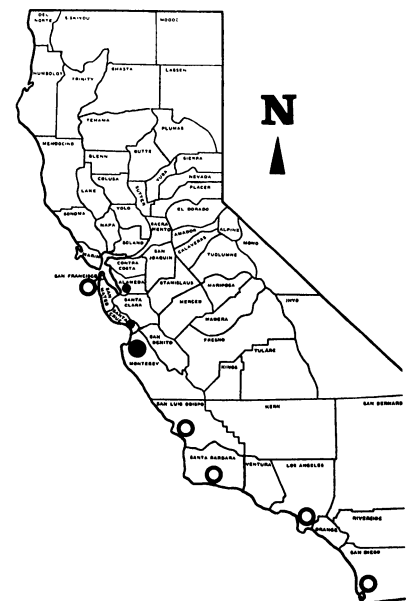


Fig. 1. Eight counties in California where pitch canker has been confirmed by isolation and subsequent verification of pathogenicity of *Fusarium subglutinans* f. sp. *pini*. Solid circles indicate counties where pitch canker has been confirmed in two or more sites, and open circles indicate where a single infection site or tree has been confirmed. Circle sizes vary only to delineate counties.

paraconfusus Lanier, respectively), the Monterey pine twig beetle (*Pityophthorus carmeli* Swaine), the Monterey pine weevil (*Pissodes radiatae* Hopkins), the Monterey pine cone beetle (*Conophthorus radiatae* Hopkins), *Hylastis nigrinus* Mannerheim, the western pine spittle bug (*Aphrophora permutata* Uhler), and the western pine leafhopper (*Koebelia californica* Baker). Pathogenic isolates of *F. subglutinans* were also recovered from several insects that are not known to feed on pine and were identified only to family, including flies (Muscidae), wasps (Vespidae), and beetles (Eucinetidae). Sixty-three pine

isolates of *F. subglutinans* from Florida, North Carolina, and Texas also were virulent on Monterey pine in the greenhouse pathogenicity test (Table 2).

Twenty-one of 23 isolates of *F. subglutinans* from nonpine hosts (sorghum, corn, sugarcane, rice, pineapple, and dracaena) from a number of geographic locations (Brazil, India, Taiwan, and eastern and western United States) were nonpathogenic on Monterey pine in greenhouse pathogenicity tests. Two rice isolates, FK343 and FK344 from Taiwan, were intermediate in virulence. Based on four inoculation experiments, FK343 and FK344 had mean disease ratings of 2.8 and 3.1, respectively.

Six pine isolates, two insect isolates, and two air sample isolates of *F. proliferatum* were tested and found to be nonpathogenic on Monterey pine, as were two pine isolates of *F. moniliforme*. Two nonpine isolates of both *F. proliferatum* and *F. moniliforme* also were nonpathogenic on Monterey pine.

Pathogenicity tests on a subsample of colonies of *F. subglutinans* from air isolation plates indicated that most, if not all, colonies of *F. subglutinans* were the pitch canker pathogen.

Field experiments. The trees inoculated in experiment A were 15- to 25-yr-old planted Monterey pines with different levels of disease (Table 3). No cankers developed as a result of mature or immature cone inoculations. The majority (> 50%) of both inoculated and noninoculated immature cones aborted and dehisced, presumably in response to wounding injury. No wounded uninoculated controls of any tissue developed cankers for any of the three inoculation dates.

Cankers developed on all inoculated branch tips, branches, and boles (Table 4). The branch and bole cankers were elliptical, usually slightly sunken, and heavily resin-soaked at the center, i.e., the point of inoculation (Fig. 2). Canker margins could be readily delineated on branches and boles after the bark was removed. Isolations were made from the margins of several branch and bole cankers. *F. subglutinans* was recovered from all branch cankers and from five of eight bole cankers. For any given bole canker, the pathogen was recovered from less than 10% of the tissue pieces sampled.

Branch tip cankers progressed fastest in the March inoculations and slowest in the November inoculations. However, the time of inoculation was not a significant factor ($P = 0.05$) in overall disease development on branch tips, branches, or bole inoculations. There was a significant block (tree) effect on branch tip disease ratings (Table 5) and canker size (Table 4) in experiment A, reflecting the influence of individual trees on disease development. Inoculated branches of trees 1 and 3 developed small-

er cankers than branches of trees 2, 4, and 5 for all three inoculation dates (Table 4). For the bole inoculations, the smallest cankers developed on trees 1 and 4 for all inoculation dates (Table 4). Overall, trees 1, 3, and 4 had the fewest infections at the onset of the experiment (Table 3). Tree 2 was the most heavily infected at the onset of the experiment (Table 3), and the largest branch and bole cankers for most inoculation dates occurred on this tree.

At the start of experiment B, three of the six inoculated trees had a large number of infected branches, whereas the other three had little or no disease and were considered putatively resistant (Table 3). One of the putatively resistant trees, tree 3, developed a substantial number of cankers (40–50%) during the course of the experiment, but no other trees showed a dramatic increase in severity during the experiment. Excluding tree 3, there were clear differences

between the susceptible and putatively resistant trees in the rate of canker development in branch tips and in the size of cankers in both small and large branches (Table 6). The mean canker size on the susceptible trees was more than twice that on the putatively resistant trees for both the small and large branch inoculations (Table 6). There was a significant block (tree) effect on both canker length and width in experiment A ($P = 0.004$ and 0.033 , respectively) and experiment B ($P = 0.002$ and 0.005 , respectively).

DISCUSSION

The pitch canker pathogen has undergone a number of name changes since it was first identified in 1946 (20). The initial report of this disease described the fungus as an unidentified species in the *Fusarium* section *Liseola*. Snyder et al. (33) later described the pitch canker pathogen as *Fusarium lateritium* Nees. Be-

Table 2. Isolates of *Fusarium subglutinans* virulent on Monterey pine in greenhouse pathogenicity tests

State	County	Site	Host pine	No. of isolates tested	Estimated no. of infected trees in sample area	
California	Alameda	Holiday Inn	Monterey	2	20	
		San Lorenzo	Monterey	7	>100	
		Lake Chabot	Monterey	2	2	
		Hayward	Monterey	3	10	
		Union City	Monterey	1	10	
	Los Angeles	Torrance	Monterey	6	50	
		Monterey	Salinas	Monterey	9	10
			Castroville	Monterey	1	5
	San Diego	Escondido	Aromas	Monterey	1	1
			Monterey	8	10	
	San Francisco		Monterey	1	1	
	San Luis	Nipomo	Monterey	1	1	
	Santa Barbara		Monterey	Monterey	6	14
			Canary Island		1	1
			Bishop		4	6
			Monterey	Monterey	15	>100
			Capitola	Monterey	3	>50
			Emeline	Aleppo	4	5
			Monterey	Monterey	5	>100
			Soquel	Monterey	2	>100
			Watsonville	Monterey	3	>100
			Aptos	Bishop	1	?
			Monterey	Monterey	1	?
			Seacliff	Bishop	1	5
			Monterey	Monterey	1	>100
	Davenport	Monterey	1	1		
	Scotts Valley	Monterey	2	2		
Subtotal				92		
All sites				Insects	36	
				Air samples	39	
Total				167		
Florida		Gainesville	Slash	10		
		Volusia	Slash	10		
		Volusia	Slash	7		
		Franklin	Slash	26		
North Carolina		Loblolly		5		
		Loblolly		4		
Texas		Loblolly		1		
		Loblolly		1		
Total				63		

cause isolates of *F. lateritium* recovered from pine were pathogenic on pine and nonpine isolates were not, Snyder et al (33) proposed that isolates pathogenic to pine be given a forma specialis designation of *F. lateritium* (Nees) emend. Snyder & Hans. f. sp. *pini* Hepting. The pathogen was subsequently identified as *F. moniliforme* Sheldon var. *subglutinans* Wollenw. & Reinking (12,22) and was more recently recognized as a distinct species, *F. subglutinans* (Wollenweb. &

Reinking) P.E. Nelson, T.A. Toussoun, & Marasas (29). The species designation was based primarily on the presence of polyphialides and production of microconidia in false heads rather than in chains.

We tested 269 isolates of *F. subglutinans* or closely related species and found only isolates of *F. subglutinans* from pine (or from air or insect samples close to diseased pine trees) were virulent on Monterey pine in greenhouse path-

ogenicity tests. All isolates of *F. subglutinans* from hosts other than pine, as well as pine and nonpine isolates of *F. moniliforme* and *F. proliferatum*, were avirulent or weakly virulent and considered nonpathogenic on Monterey pine. However, two rice isolates of *F. subglutinans* were intermediate in virulence on Monterey pine. Barrows-Broadus and Dwinell (2) also noted that isolates of the pitch canker pathogen could cause slight to moderate decay of artificially inoculated gladiolus corms. On the basis of our findings, of similar findings by others (12,13), and of the lack of polymorphisms in mtDNA (11; J. C. Correll, T. R. Gordon, and A. H. McCain, unpublished), isolates pathogenic to pine appear to represent a distinct subpopulation within *F. subglutinans*. Accordingly, we propose that this pathogen be recognized as a separate forma specialis, *Fusarium subglutinans* f. sp. *pini*.

Field inoculation experiments indicated that all ages of branch tissue and boles of Monterey pine were susceptible to infection. Although inoculation of cones did not result in infection, the pathogen has been recovered from numerous naturally infected cones in the field. Furthermore, field observations indicated that cone whorls often were associated with the appearance of very small, newly developing cankers. It is possible that infections at the proximal end of

Table 3. Age, height, diameter at breast height, and approximate disease severity of trees used in field inoculation experiments

Tree no.	Age (yr)	Height (m)	Diameter (cm)	Percentage of branches with cankers ^a	
				Nov. 1987	June 1989
Experiment A					
1	25	20	69	10	10
2	25	18	71	85	85
3	25	24	71	20	20
4	15	10	28	15	15
5	25	14	51	25	25
Experiment B					
1	25	19	61	5	10
3	17	10	28	10	45
5	20	17	56	0	5
2	20	18	53	40	40
4	15	13	58	85	85
6	21	10	41	70	70

^a Disease severity was estimated by examining each tree and approximating the percentage of branches infected throughout the canopy. Only tree 3 from experiment B had a substantial increase in disease severity over the duration of the two experiments.

Table 4. Canker size of branch and bole inoculations, experiment A

Inoculation date	Elapsed time (mo)	Canker size (cm) ^a					Mean canker size (cm)
		Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	
Branch inoculations							
November 1987	19	7 × 2	18 × 4	5 × 1	19 × 6	...	12.3 × 3.3
March 1988	15	8 × 2	20 × 7	10 × 2	11 × 3	13 × 3	12.4 × 3.4
July 1988	13	5 × 1	14 × 3	6 × 2	9 × 3	7 × 3	8.2 × 2.4
Bole inoculations							
November 1987	19	11 × 3	25 × 6	28 × 9	15 × 4	22 × 7	20.2 × 5.8
March 1988	15	10 × 2	32 × 6	20 × 7	16 × 3	21 × 4	19.8 × 4.4
July 1988	13	2 × 1	13 × 4	21 × 5	10 × 4	24 × 5	14.2 × 3.8

^a All inoculations were measured in June 1989. Each number is the mean of two inoculations per tree. All uninoculated controls and inoculated cones remained uninfected.

Table 5. Disease rating of branch tip inoculations, experiment A

Inoculation date	Elapsed time (mo)	Disease rating ^a					Mean disease rating
		Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	
November 1987							
	5	1.5	3.0	1.0	1.0	1.0	1.5
	7	1.5	5.0	1.0	3.5	5.0	3.2
	9	3.0	5.0	3.0	5.0	5.0	4.2
March 1988							
	1	1.5	2.0	1.5	1.5	2.0	1.7
	2	3.0	4.5	4.5	4.5	5.0	4.3
	5	4.0	5.0	5.0	5.0	5.0	4.8
July 1988							
	1	1.0	1.0	1.0	1.0	1.0	1.0
	4	2.5	4.5	3.0	4.5	5.0	3.9
	9	3.5	5.0	3.5	5.0	5.0	4.4

^a Each number is the mean of two inoculations per tree. Rating is on a scale of 0-5, where 0 = healthy and 5 = girdling lesion with most (> 75%) of the needles distal to the inoculation point necrotic. All uninoculated controls remained uninfected.



Fig. 2. Vertical, elliptical canker development on the bole of a 25-yr-old Monterey pine tree 19 mo after inoculation with *Fusarium subglutinans*. Tree diameter at the point of inoculation (arrow) is approximately 40 cm. Heavy resinosis at the canker center and resin-soaked wood at the canker margin are apparent.

Table 6. Disease rating of branch tip inoculations and canker size of branch inoculations, experiment B^a

Elapsed time (mo.)	Resistant trees				Susceptible trees			
	No. 1	No. 3	No. 5	Mean	No. 2	No. 4	No. 6	Mean
Branch tip inoculation disease rating ^b								
2	1.2	2.6	1.0	1.6	1.8	1.8	2.6	2.1
3	2.2	4.2	1.4	2.6	2.6	3.5	4.2	3.4
5	4.0	5.0	3.2	4.1	4.4	5.0	5.0	4.8
Small branch inoculation canker size (cm) ^c								
13	5 × 1	20 × 6	7 × 3	11 × 3	21 × 5	20 × 7	22 × 6	21 × 6
Large branch inoculation canker size (cm) ^c								
13	7 × 2	22 × 6	10 × 2	13 × 4	29 × 8	24 × 7	25 × 6	26 × 7

^a Trees 1, 3, and 5 were tentatively identified as resistant and trees 2, 4, and 6 as susceptible at the onset of the experiment.

^b Rating is on a scale of 0-5, where 0 = healthy and 5 = girdling lesion with most (> 75%) of the needles distal to the inoculation point necrotic. Each rating is the mean of five inoculations per tree.

^c Each number is the mean of five inoculations per tree.

cones by mechanical or insect wounds would not cause cone abortion, as many of our distal end inoculations did and could potentially lead to branch cankers.

The severity of branch and bole cankers that developed after field inoculations suggests that pitch canker could be the primary cause of tree mortality in locations in California where the disease has been severe. Large cankers with severe resinosis were produced in less than 2 yr after branch and bole inoculations (Tables 4-6, Fig. 2).

Airborne inoculum of *F. s. pini* was present at varying levels throughout the year. Although direct comparisons are difficult, airborne inoculum levels in California appear to be less than those recorded in North Carolina (25) or Florida (9), where isolation plates were exposed for 0.5 or 6 hr, respectively. None of the 50 noninoculated wounds in the field experiment became infected, even though airborne inoculum was present throughout the year. The recovery of *F. s. pini* from many insects capable of feeding on and wounding Monterey pine indicates that insects could be very important in the epidemiology of this disease in California. Several species of *Ips* may be particularly important in vectoring the pitch canker pathogen, as they are closely associated with diseased Monterey pine trees in this area (17). However, many of the insects that were contaminated with *F. s. pini* may have contacted the fungus incidentally.

In areas of California with a high disease incidence, it is common to see apparently healthy Monterey pine trees adjacent to severely infected Monterey pines. Field inoculation experiments in two different locations revealed highly significant differences in the extent of canker development among individual inoculated trees. These results may reflect genetic differences among Monterey pine trees in their susceptibility to pitch canker. However, other factors such as insect feeding preference, insect population densities, and site conditions

also are likely to influence disease severity under field conditions. Consequently, to establish the basis for differences in susceptibility to pitch canker in Monterey pine, experiments conducted in a controlled environment will be required.

The pitch canker pathogen apparently is well established in several central and southern coastal counties of California. *F. s. pini* has been recovered from over 20 locations in eight counties in the state between 1986 and 1989. Although Monterey pine is the primary host, the pathogen has also been recovered from symptomatic bishop, Aleppo, and Canary Island pines. Disease incidence and severity vary considerably but appear to be the highest in Santa Cruz County, where the disease was first observed (28).

In California, pitch canker has caused damage primarily to Monterey pine planted in roadway right-of-ways and landscape settings. The disease also has been identified on Monterey pine in several tree nurseries and Christmas tree farms. Pitch canker disease has not yet been detected in any of the native stands of Monterey pine in California or in any locations outside the planted range of Monterey pine in the state. However, over 25 pine species, either native to or planted in California, were susceptible to this pathogen under greenhouse conditions (28; A. H. McCain, unpublished). Consequently, pitch canker could be a threat to pines throughout the state.

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