PINE

CONTAMINATION OF *PINUS RADIATA* SEEDS IN CALIFORNIA BY *FUSARIUM CIRCINATUM*

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The pitch canker fungus, *Fusarium circinatum* (= *F. subglutinans* f. sp. *pini*), causes several serious diseases of pines. The pathogen infects a variety of vegetative and reproductive pine structures at diierent stages of maturity and produces a diversity of symptoms. In addition to producing resinous cankers on the woody vegetative structures of its pine host, the causal fungus causes the mortality of female flowers and mature cones, deteriorates seeds of several pine species, and can cause mortality of seedlings in nurseries. Since 1986, pitch canker has been epidemic *in* California on *Pinus radiata* (Monterey pine) and has all the earmarks of an introduced disease.

Recovery from seeds. In September 1998, ninety-five Monterey pine cones were collected at 11 locations in the central coastal region of California between Half Moon Bay and Cambria (Table 1). The seeds were extracted from the cones and the level of seed contamination was determined by plating 20 seeds/cone on a *Fusarium*-selective media. The percentage of full seeds was determined by radiographing seeds pooled by location. Germination rate was also assessed on the geographical collections. Fifteen representative colonies per location were identified to species and tested for pathogenic&y by inoculating shoots of Virginia pines *(P. virginiana).*

In the cones collected in the Carmel Valley, 99% of the seeds were contaminated by *F. circinatum* (Table 1). Contaminated seeds were also extracted from cones collected in Santa Cruz, Monterey, Half Moon Bay, Cambria, Prunedale, and along the Big Sur. All tested isolates were determined to be the pitch *canker* fungus. *Seed* contamination by *F. circinatum was* not present in cone collections at Point Año Nuevo, Sunset Beach State Park Salinas, and Point Lobos State Reserve. The percentage of full seeds averaged 80% among all seed collections, and seedlots from Sunset Beach State Park (62%) and the Big Sur (44%) had the fewest full seeds. Germination for the 11 geographical seed lots averaged 30% (range 9-50%).

Pathogenicity. The pathogenicity of *F. circinatum* from cones and seeds of Monterey pine in California was tested by inoculating 14-month-old Monterey pine seedlings with 9 geographical isolates. Isolates of *F. circinatum* were also included from Douglas-fir (*Pseudotsuga* sp.) @F-l) in California, pines in the southeastern U.S. (D-27, D-19), and *P. radiata seedlings in Spain* (M-8490, M-8488), as well as isolates of *Fusarium subglutinans* from mango inflorescences in Florida (M-361 1, M-3622). Seedlings @/isolate) were inoculated by puncturing the bark of the main stem with a needle contaminated by the pathogen. Nail polish was used to mark the inoculation site. The seedlings were examined weekly, and data obtained on rate at which isolates girdled the main stem and proximal canker length (cambial necrosis below point of inoculation).

All the isolates of *F. circinatum* developed girdling cankers on the Monterey pine seedlings within 23 days after inoculation. Proximal Canker length was not related to isolate and averaged 36.6 mm. The isolates of *F. subglutinans* from mango were nonpathogenic.

Internal vs External **Contamination.** Two experiments were conducted to determine if the contamination was internal or external. In the first, pooled seeds were treated in a 30% solution of hydrogen peroxide for 15 min, rinsed in sterile water, and then plated on PCNB agar. In the second, pooled seeds were surface-sterilized in 70% EtOH for 15 sec, followed by a 60 sec soak in 1% sodium hypochorite (NAOCL), and two water rinses. Each experiment included a water control. In both experiments, 70 seeds per treatment were placed on a *Fusarium*-selective media (10 seeds/plate).

In both experiments, all of the control seeds were contaminated by the pitch canker fungus. Only 4% of those treated with hydrogen peroxide yielded *F. circinatum*. The seeds treated with hydrogen peroxide, from which the fungus was isolated, were 'pops". The pathogen was not isolated from seeds that had been surfaced sterilized with EtOH/NAOCL. This data suggests that seed contamination by the pitch canker fungus was largely external.

Seed Treatments. External contamination of pine seeds by fungal pathogens can be eradicated by appropriate seed treatments. Hydrogen peroxide, for example, shows promise as a seed disinfectant. As noted previously, 30% hydrogen peroxide may prove beneficial for the reduction or elimination of the pitch canker fungus from Monterey pine seeds. A recent study on percent hydrogen peroxide concentration x time suggests that Monterey pine seeds can be decontaminated by treatment in a 20% hydrogen peroxide solution for 5 min (unpublished data). Benomyl and Thiram have also shown benefit as seed treatments.

In the spring of 1999, a study was established in a sandy loam soil in raised-beds to determine the efficacy of several seed treatments. Seeds of slash (*P. elliottii var. elliotti*), longleaf (*P. pautris*), and Monterey pine were artificially-contaminated with *F. circinutum, dried, and* subsequently treated with hydrogen peroxide (5% for 55 min and 30% for 15 mm), Benomyl or Thiram. Uninoculated seeds were similarly treated. Each treatment combination was replicated 4 times (100 seeds/replicate). Data on germination and seedling mortality were recorded periodically for 5 mo. Seedling mortality was a function of the interaction between seed contamination and host (Table 2). Basically, there was significantly more post-emergence damping-off in Monterey pine seedlings *grown* from *F. circinatum*-contaminated *seeds than* from uncontaminated seeds. There was no significant post-emergence damping-off in slash or longleaf pine seedlings grown from contaminated seeds. Furthermore, differences in seed treatments were not statistically significant.

Nurseries. There is little empirical data linking seed contamination by *F. circinutum with seedling* cankers that occurs in nursery beds and on outplanted sites. Current data suggests that the major result of seed contamination by the pitch canker fungus is pre- and post-emergence damping-off. Information on the relationship between seed contamination and disease will help nurseries develop control strategies.

Table 1. Contamination of seeds of Monterey pine by *Fusarium circinatum in* California.

Collection location	Seed contamination	Full seeds	Seed Germination
	%	%	%
Cannel Valley 99 a	76	20	
Half Moon Bay	54 b	90	44
Cambria	40 bc	84	48
Santa Cruz	34 bcd	92	10
Monterey	34 bcd	84	44
Big Sur	24 cd	44	17
Prunedale	12 cd	84	24
Point A_o Nuevo	Od	84	18
Sunset Beach State Park	0 d	62	9
Salinas Od	88	50	
Point Lobos State Reserve	0 d	86	42

Cones were collected in September 1998 and the seeds extracted. Twenty seeds/cone were plated on *Fusarium-selective* media. The percentage of seeds contaminated by *F. circinatum was* determined and analysed by one-way ANOVA. Means followed by the same letter within % contaminated are not significantly different. The percent full seeds was determined by radiography. Percent germination was determined for 200 seeds per location.

Table 2. The effect of the seed contamination x host interaction on post-emergence damping-off.

Seed Contamination by *F. circinatum*	Pine host	% Mortality
Inoculated	Monterey	14.3 a
	Slash	1.8 b
	Longleaf	2.4 b
Uninoculated	Monterey	4.2 b
	Slash	1.8 b
	Longleaf	1.6 b

Means followed by the same letter are not significantly different.