Association of the Pitch Canker Fungus with Cones and Seeds of Pines

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ABSTRACT

The pitch canker fungus, Fusarium subglutinans f. sp. pini, causes the mortality of female flowers and mature cones, and can infect and destroy gametophyte tissues of seeds of several pine species in the southeastern U.S. The fungus can also be associated with the seed coats of apparently healthy, viable pine seeds. The pitch canker fungus was isolated from wash water from Monterey pine cones at all sampling locations in central coastal California, and the frequency of isolations was greatest in those areas, except Sunset Beach State Park, where the disease was the most common, and least where evidence of disease is low or absent. Assaying Monterey pine cones for surface contamination by F. subglutinans f. sp. pini may be useful in monitoring the inoculum level in geographical locations. The incidence of contamination of Monterey pine seeds also varied significantly by location. The contamination of longleaf, shortleaf, and Monterey pine seeds by the pitch canker fungus appears to be primarily external, although the presence of some internal contamination in some seed lots cannot be dismissed. External contamination of pine seeds can be reduced or eliminated by appropriate seed treatments.

“Pitch canker” incompletely describes the variety of damage caused by Fusarium subglutinans (Werlenw. & Reinking) Nelson, Tousoum., and Marasas f. sp. pini (= F. circinatum Nirenberg and O’Donnell) to pines. When the pathogen infects the woody vegetative structures of its pine host, the host-pathogen interaction causes resinous cankers, and the resultant disease is referred to as pitch canker. The pitch canker fungus also causes the mortality of female flowers and mature cones, deteriorates seeds of several pine species, and can cause mortality of pine seedlings in nurseries (Barrows-Broadus 1987; Blakeslee 1980; Dwinell et al. 1985). The involvement of insects, interaction with other pine diseases, and the marked influence of biotic and abiotic factors can greatly influence the incidence and severity of infection by F. subglutinans f. sp. pini (Barrows-Broadus 1987; Dwinell et al. 1985).

Southern pines Miller and Bramlett (1978) established that the pitch canker fungus was pathogenic to both first- and second-year female strobili of slash (Pinus elliottii Engelm. var. elliottii) and loblolly (P. taeda L.) cones inoculated with F. subglutinans f. sp. pini. Inoculated cones became necrotic, and the pitch canker fungus could be isolated from the cone scales, the axis, and the seeds. Dwinell and Fehrlich (1997a) isolated the pitch canker fungus from the surface and interior of shortleaf pine (P. echinata Mill.) cones collected in a North Carolina seed orchard. They found no apparent correlation between necrotic cones with external wounds caused primarily by insects and isolation of the fungus from internal tissues. Barrows-Broadus (1987) reported that infected loblolly pine cones tended to be misshapen and smaller than normal, and some cones have a necrotic tip characterized by internal resin pockets. Mycelium of the causal fungus has been observed on the outer surfaces of badly deteriorated cones of slash and loblolly pines. The mode of entry of F. subglutinans f. sp. pini, a wound parasites (Dwinell et al. 1985), into cones of southern pines is currently not known.

Miller and Bramlett (1978) isolated the pitch canker fungus from gametophyte and embryo tissue of slash and loblolly pine. Radiographs of seeds in advance stages of disease may show deterioration of the embryo and a gametophyte which has shrunked away from the seed coat. Microscopic examination may reveal the presence of hyphae throughout these seeds (Barrows-Broaddus 1987). Radiographs, however, will not reveal external seed contamination. In many pine species seed contamination may be largely restricted to the seed coat. *Fusarium subglutinans* f. sp. *pini*, for example, was isolated from an average of 61% of the freshly extracted shortleaf pine seeds; but only 1.6% of the seeds were infested internally (Dwinell and Friedrich 1997a). The pathogen appears to be primarily associated with the seed coat on longleaf pine (*P. palustris* Mill.) seeds, and infection of the endosperm and embryos is rare (Dwinell and Friedrich 1997b). The extent of internal and/or external seed contamination varies by southern pine species (Dwinell and Friedrich 1999).

**Monterey pine** Because cankers on Monterey pine frequently occur at branch and cone whorls, there has been considerable interest in the possible role of cone beetles, particularly *Conophthorus radiatae* Hopkins and *Ernobius punctulatus* Fall. *Conophthorus radiata* attack healthy cones; but *E. punctulatus* lives in dead cones and is a common associate of *C. radiatae*. Hoover et al. (1995) reported that 21% of *C. radiatae* and 30% of *P. punctulatus* adults dissected from cones carried spores of the pitch canker fungus. There was considerable seasonal variation in the number of contaminated adult *C. radiatae* and *E. punctulatus* emerging from cones. This may explain why I isolated the pitch canker fungus from only 5% of the 100 adults I removed from dissected Monterey pine cones in 1988–89 (unpublished data). Hoover et al. (1996) demonstrated that *C. radiatae* could transmit the pitch canker fungus to healthy cones and that the fungus subsequently colonized the cones. Cones colonized by *C. radiatae* wither and die. The relationship between the colonisation of Monterey pine cones by *C. radiatae* and incidence of branch cankers at cone clusters has not been demonstrated.

In 1987, Broadus (Brenau University, Gainesville, GA) and I isolated the pitch canker fungus from the cones and seeds of Monterey pine growing in the Santa Cruz area. The fungus, however, was not recovered from stored seedlots from California and New Zealand that predated the pitch canker outbreak (unpublished data). In 1987, Ferrin et al. (1991) collected 104 cones from 26 Monterey pines in the Santa Cruz area and found that 62% of them contained at least one contaminated seed. Furthermore, their data indicates that 14% of the Monterey pine seeds were contaminated externally, and 11% were infested internally.

In September 1998, I collected mature cones from 95 Monterey pine trees at 11 locations in central coastal California between Half Moon Bay and Cambria (Table 1). The surface of the cones were flooded with 20 ml merthiolate distilled water and a 1 ml suspension of each of three dilutions (1:20, 1:200, and 1:2000) was plated on a *Fusarium*-selective medium. The number of colony-forming units (cfu)/cone of the pitch canker fungus were determined. Fifteen representative colonies per location were identified for species and pathogenicity determined by inoculating shoots of Virginia pines (*P. virginiana* Mill.) growing under greenhouse conditions (Dwinell and Friedrich 1997a). All representative isolates were the pitch canker pathotype of *F. subglutinans*. By far, the greatest number of cfu of *F. subglutinans* f. sp. *pini* were for those collected in Santa Cruz — 234,800. Carmel, Monterey, and Point Año Nuevo averaged 13,153 cfu/cone. The other seven locations averaged 446 cfu/cone. Interestingly, the pitch canker fungus was found in locations, such as Point Lobos State Reserve and Big Sur (Highway 1 between Point Lobos State Reserve and Cambria) that have little or no disease. This cone wash method may be useful for monitoring the inoculum level of the fungus in geographic locations.

The level of contamination of the seeds from the 95 Monterey pine cones collected in September 1998 was determined by plating 20 seeds/cone on a *Fusarium*-selective medium. Fifteen representative colonies per location were identified to species and tested for pathogenicity by inoculating shoots of Virginia pines (Dwinell and Friedrich 1997a). All representative isolates were confirmed to be *F. subglutinans* f. sp. *pini*. The percentage of full seeds was determined by radiographing seeds pooled by location. Germination rate was also assessed for the pooled seeds. In the Carmel area, 99% of the seeds were contaminated (Table 1). Contaminated seeds were also extracted from cones collected in Santa Cruz, Monterey, Half Moon Bay, Big Sur, Cambria, and Prunedale. Seeds tested from Point Año Nuevo, Sunset Beach State Park, Salinas, and Point Lobos State Reserve were not contaminated by the pitch canker fungus. The percentage of full seeds averaged 80%, with the seedlots from Sunset Beach State Park (62%) and the Big Sur (44%) having the fewest full seeds. Germination for the 11 seed lots averaged 30% (range 9–50%). To determine if the contamination was internal or external, pooled seeds were treated in a 30% solution of hydrogen peroxide for 15 minutes or a water control. Seventy seeds per treatment were plated on a *Fusarium-
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cone were 

TABLE 1. Association of *Fusarium subglutinans* f. sp. *pini* (FSP) with cones and seeds of Monterey pine in central coastal California

<table>
<thead>
<tr>
<th>Location</th>
<th>Cones 1</th>
<th>Seeds 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>FSP (cfu/cone)</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>10</td>
<td>234 800 a</td>
</tr>
<tr>
<td>Carmel</td>
<td>10</td>
<td>21 600 b</td>
</tr>
<tr>
<td>Monterey</td>
<td>10</td>
<td>10 800 b</td>
</tr>
<tr>
<td>Point Año Nuevo</td>
<td>10</td>
<td>690 b</td>
</tr>
<tr>
<td>Half Moon Bay</td>
<td>10</td>
<td>696 c</td>
</tr>
<tr>
<td>Sunset Beach S.P.</td>
<td>5</td>
<td>588 c</td>
</tr>
<tr>
<td>Big Sur</td>
<td>10</td>
<td>508 c</td>
</tr>
<tr>
<td>Salinas</td>
<td>5</td>
<td>480 c</td>
</tr>
<tr>
<td>Cambria</td>
<td>10</td>
<td>422 c</td>
</tr>
<tr>
<td>Point Lobos S.B.</td>
<td>10</td>
<td>341 c</td>
</tr>
<tr>
<td>Prunedale</td>
<td>5</td>
<td>88 c</td>
</tr>
</tbody>
</table>

1 Cones were collected in September 1998. The cones were flooded with 20 ml sterile distilled water and a 1 ml sus- 
pension of a each of three dilutions (1:20, 1:200, and 1:2000) was plated on a *Fusarium*-selective medium. The 
cfus/curve was determined and square root transformed data analysed by one-way analysis of variance. Means of 
untransformed data followed by the same letter within a column are not significantly different.

2 Seeds were extracted from the cones. Twenty seeds/cone were plated on a *Fusarium*-selective medium. Percentage of 
seeds contaminated by *F. subglutinans* f. sp. *pini* (FSP) was determined and analysed by one-way analysis of 
variance. Means followed by the same letter within % contaminated are not significantly different. Percentage full 
seed was determined for 50 seeds per location by radiography. Percentage germination was determined for 200 
seeds per location.

selective medium. All of the control seeds were 

Seeds treatments: External contamination of pine 

outplanted sites. In a greenhouse study, Dwinell and 

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**Question and Answer**

**Sharon Clark:** In the first study you talked about cones that you collected from California. How did you handle the cones from each location, and how were the cones opened to collect the seed?

**Dave:** Each of the cones was individually handled. We put them in boiling water for 30 seconds and then let them dry and open up in a paper bag. I believe this is the traditional or normal method for collecting seed from Monterey pine.

**Bro Kinloch:** Curious to know why you don’t take the seed coats off and presterilize them.

**Dave:** We’ve done some of that and find it doesn’t buy us anything. We used to crush the seed, but we find we get the same results just by plating them whole, the fungus just grows out of the seed without any problem. Early on we did some excising work, but we find at least for these species, it’s not necessary.

**Scott Templeton:** I’m detecting some policy implications which might be different from those of David and Andrew. Are you saying you can control the spread of the disease with chemical treatments of parts of the trees?

**Dave:** No I’m not saying that. I don’t know where you heard that.

**Scott:** Because you said the fungus mostly exists on the surface.

**Dave:** On the seed, only on the seed. I’m saying I can treat the seed. This is important, for example I have a group from China who want to import some southern pine seed, particularly longleaf pine. If they wanted to bring in clean seed, they can hydrogen peroxide or treat it with Chlorox they can import clean seed. It’s only the seed.

**Mike Wingfield:** Dave you’ve got me confused here. Previous reports have shown that the pitch canker fungus is found to be internally seed borne.

**Dave:** Possibly slash and possibly lobolly. I’m only talking about longleaf, shortleaf and Monterey.

**Mike:** Why would those be different?

**Dave Wood:** It’s in Monterey as well, Andrew will give a talk on that.

**Dave:** That’s fine, I’m only presenting my data.

**Mike:** This is really a very important issue.

**Dave:** It varies, it’s different for different species. George and I were talking about this the other day in slash pine. In slash it tends to be more internally contaminated and possibly to a certain degree lobolly. But as far as Monterey, longleaf, and shortleaf, so far it’s been external contamination we’ve been dealing with.

**Mike Carson:** Dave, in the break earlier you commented on the wind dissemination of spores versus an insect vector. Is the cone wash information that you presented evidence for that?
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