PINE

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

David Dwinell

USDA Forest Service, Southern Research Station, Athens, GA

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* (Wollenw. & Reinking) Nelson, Toussoun, 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-Broaddus 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-Broaddus 1987; Dwinell et al. 1985).

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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 Fraedrich (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-Broaddus (1987) reported that infected loblolly pine cones tended to be misshapen and smaller than normal, and some cones have a necrotic tips characterised 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 parasite (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 shrunken 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 Fraedrich 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 Fraedrich 1997b). The extent of internal and/or external seed contamination varies by southern pine species (Dwinell and Fraedrich 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, ***E. 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 el al. (1996) demonstrated that ***C.*** *radiatae* could transmit the pitch canker fungus to healthy cones and that the fungus subsequently colonised the cones. Cones colonised ***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, Broaddus (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. (199 1) 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 sterile 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 to species and pathogenicity determined by inoculating shoots of Virginia pines (*P. virginiana* Mill.) growing under greenhouse conditions (Dwinell and Fraedrich 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*/cone 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 Fraedrich 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-***

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

Location	Cones[1]		Seeds[2]		
	Number	FSP (cfu/cone)	Contaminated (%)	Full (%)	Germination (%)
Santa Cruz	10	234 800 a	34 bcd	92	10
Carmel	10	21 600 b	99"	76	20
Monterey	10	10'880 b	34 bcd	84	44
Point Aiio Nuevo	10	6980 b	0 d	84	la
Half Moon Bay	10	696 c	54 b	90	44
Sunset Beach S.P.	5	588 c	0 d	62	9
Big Sur	10	508 c	24 cd	44	17
Salinas	5	480 c	0 d	88	50
Cambria	10	422 c	40 b	84	48
Point Lobos S.R.	10	341 c	0 d	86	42
Prunedale	5	88 c	12 cd	84	24

1 Cones were collected in September 1998. The cones were flooded with 20 ml sterile distilled water and a 1 ml suspension of a each of three dilutions (1:20, 1:200, and 1:2000) was plated on a *Fusarium*-selective medium. The cfu/cones 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 contaminated with the pitch canker fungus, but only 4% of those treated with hydrogen peroxide yielded the pitch canker fungus. The treated seeds from which the fungus was isolated were "pops". These data suggest that seed contamination by the pitch canker fungus was largely external. Seed contamination may have partially resulted from the opening and closing of the mature Monterey pine cones with changes in temperature and humidity.

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 (Bamett 1976; Dwinell and Fraedrich 1997b). Dwinell and Fraedrich (1997b) reported that longleaf pine seeds can be decontaminated by treatment with a 30% hydrogen peroxide solution for 55 minutes. They also reported that a 30% hydrogen peroxide solution for 15 minutes would decontaminate shortleaf pine seeds (Dwinell and Fraedrich 1999). As noted previously hydrogen peroxide may prove beneficial for the reduction or elimination of the pitch canker fungus from Monterey pine seeds.

Nurseries There is little empirical data linking seed contamination by *F. subglutinans* f. sp. *pini* with seedling cankers that occur in nursery beds and on outplanted sites. In a greenhouse study, Dwinell and Fraedrich (1999) artificially contaminated Monterey and slash pine seeds with an isolate of the pitch canker fungus. Of the total container-sown seeds, 57% and 30%, respectively, of the Monterey and slash pine seedlings had damped-off after emergence and 22% of the Monterey pine seeds had damped-off prior to emergence. Preliminary data suggest that the major result of seed contamination by the pitch canker fungus is pre- and post-emergence damping-off.

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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 loblolly. 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 loblolly. 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?

Dave: I think so. The 1989 bark wash data plus the work of Correll in the early 1990s very much confirms that the fungus is getting around aerially.

Sharon Clark: Based on your radiograph experience, I've never done that, you mentioned that some species you could tell in the seeds if there was contamination, but in Monterey pine you were not able to make a determination.

Dave: Right in slash pine, but not in Monterey pine; you can't tell anything from the internal. In slash you can sometimes see some shadowing, but if you're dealing with external contamination you can't tell anything from the autoradiograph except percent full seeds.

Dave Wood: You indicated that you thought in Monterey pine that the fungus is growing upstream?

Dave: No I don't think it's been demonstrated which way it's going. I think the evidence may suggest that it's going upstream.

Dave Wood: Well if it is, it doesn't seem to be going very far. Our observation is that it sort of stops. Would you say that's true Sharon? In lab inoculations and even tip dieback in the field when you go back, it's in the same location and is distal to a certain point, it's dead, and then no more dieing.

Dave: In my next paper and in earlier observations in 1986, we showed that the cankers extended below branch whorls. Like southern pines, Monterey pine doesn't produce any adventitious growth.

George Blakeslee: Back when we worked on pitch canker in slash pine. This was a long time ago and my memory is so weak I have trouble finding my way home at night, so don't quote me on percentages. We went to seed beds designated as high, and lifted seedlings from operational liftings and then went to low infection beds, and outplanted from these beds, and followed post planting mortality. It was dreadful, the low disease incidence beds had good survival, good growth. From the high infection level beds even though planting green healthy seedlings the mortality was very high, nearly 100%. So there is that nursery to field connection.

Dave: No I understand, that doesn't give the seed connection but it does give the nursery to field connection.

George: Yes but your comment was that it hadn't been looked at. It has been looked at, and the results were obvious. If you take seedlings that are swimming in inoculum and then take them out and plant them, they become infected and die. It was sort of a no brainer.

Dave: Of course with the longleaf work, which I didn't get into, we've tracked the fungus in longleaf nurseries. We know we get inoculum in the nurseries, if you wound them you will get cankering and death which is totally independent of what you see in seeds.