

Effects of Inoculation Treatment with *Fusarium moniliforme* var. *subglutinans* on Dieback of Loblolly and Slash Pine Seedlings

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ABSTRACT

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Pinhole or knife-slit wounds were more effective courts of entry for the pitch canker disease organism in greenhouse inoculations than wounds created either by removing needle fascicles or by clipping branches. Moist incubation for 24 hr immediately after inoculation did not enhance the ability of *Fusarium moniliforme* var. *subglutinans* to cause dieback. Inoculation of fresh wounds on loblolly and slash pine seedlings produced 78% dieback, whereas inoculation of 21-day-old wounds produced only 20% dieback.

Additional key words: *Pinus elliottii*, *P. taeda*, pitch canker disease

Pitch canker disease of southern pines, including loblolly (*Pinus taeda* L.) and slash (*P. elliottii* Engelm. var. *elliottii*) pines, is caused by *Fusarium moniliforme* Sheld. var. *subglutinans* Wollenw. & Reink. (*F. m.* var. *subglutinans*) (6,9,15). When stems of several pine species were slit-wounded with a knife and inoculated with isolates of *F. m.* var. *subglutinans*, the stem died back to the inoculation wound and the fungus was reisolated (6,15). Slit-wounded seedlings not inoculated or inoculated with isolates of other *Fusarium* species rarely had dieback (0 and 2% of 100 and 647 seedlings, respectively). Recent major outbreaks of this disease have occurred in slash pine plantations in Florida and in seed orchards of slash and loblolly pines throughout the South (2,9-11,13,14, 17,18). In loblolly pine seed orchards, the

major symptom has been dieback of terminal and lateral branches. Pitch canker disease has generally been associated with wounding either by insects or by mechanical damage. Hepting and Roth (12) indicated unbroken bark of Virginia pine (*P. virginiana* Mill.) was not susceptible to infection, whereas wounds were susceptible. Various insects, including the Nantucket pine tip moth (*Rhyaciona frustrana* (Comstock)) and the deodar weevil (*Pissodes nemorensis* Gemar), have been suggested as the wounding agents and/or the vectors for the disease (5,16). In pine seed orchards, mechanical damage caused by tree shakers or cone removal can create courts of entry (3,7-9).

Previous research has demonstrated wounds and virulent isolates of *F. m.* var. *subglutinans* are necessary for dieback of pine to occur (6,15). This study compared the effects of type of wound, age of wound, and inoculum density on dieback of loblolly and slash pines by *F. m.* var. *subglutinans* in greenhouse inoculations.

MATERIALS AND METHODS

One- to 2-yr-old loblolly and slash pine seedlings were growing in a soil:peat moss mix (1:1, v/v) in 6-in. plastic pots at the time of inoculation. All seedlings in each experiment were the same age.

Inoculum. Four isolates of *F. m.* var. *subglutinans* recovered from loblolly pine and one from slash pine were grown on PDA for 7-10 days. Water suspensions of conidia from these cultures were combined, counted with a Coulter counter, and adjusted to 10^6 spores per milliliter or to other spore concentrations for the inoculum density study. The spore suspension was sprayed to runoff on the wounded area of pine seedlings in four tests.

1. Inoculum density. Each of six spore concentrations of 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , and 10^7 conidia per milliliter and a water control was sprayed on 50 slit and 50 pinhole wounds on loblolly pine seedlings. Slit wounds were single 5-cm-long knife slits. Pinhole wounds were five holes 3-5 cm deep made within a 1-cm² area with a dissecting needle on each seedling.

2. Age of wound. Loblolly and slash pine seedlings were wounded with slit or pinhole wounds 0, 3, 7, 14, and 21 days before being sprayed with inoculum or a water control. Each wound treatment was imposed on 30 seedlings of each species for each age of wound treatment (two pine species × two wound types × five wound ages × two inocula [fungus and a water control] × 30 seedlings = 1,200 seedlings).

3. Wound types. The six wound treatments were five pinholes, five adjacent needle fascicles removed, one lateral branch clipped off to simulate cone harvest, 5-cm-long knife slit, and nonwounded (control). Seventy-five loblolly pine seedlings received each treatment followed by the inoculum spray and a 24-hr moist period.

4. Tissue age. One-year-old stem tissue and current stem tissue of loblolly pine were subjected to three wound treatments: pinholes, slit wound, or removal of six adjacent needle fascicles. Each of the three wound treatments was imposed on 40 loblolly pine seedlings of each tissue

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Table 1. Effect of inoculum of *Fusarium moniliforme* var. *subglutinans* conidia on dieback of loblolly pine seedlings

Inoculum density (conidia/ml)	Dieback ^z (%)
10 ²	23 c
10 ³	43 bc
10 ⁴	66 ab
10 ⁵	67 ab
10 ⁶	85 a
10 ⁷	79 a

^zBased on 100 seedlings per inoculum density. Values followed by the same letters are not significantly different ($P = 0.01$). Control seedlings had no dieback.

Table 2. Effect of age of wound on dieback of loblolly and slash pine seedlings after inoculation with *Fusarium moniliforme* var. *subglutinans*

Age of wound (days)	Dieback (%) ^y		
	Loblolly	Slash	Combined ^z
0	88	67	78 a
3	58	33	46 b
7	50	28	39 b
14	32	20	26 c
21	20	20	20 c

^yBased on 60 seedlings of each species at each age. Uninoculated controls had 0 and 3% mortality, respectively, for loblolly and slash pines.

^zValues followed by the same letters are not significantly different ($P = 0.01$).

Table 3. Effects of wounding treatments on dieback of loblolly pine seedlings caused by *Fusarium moniliforme* var. *subglutinans*

Wound treatment	Dieback ^a (%)	Occurrence of dieback after inoculation (wk)
		(wk)
Pinholes	63	7.7
Slit	55	7.0
Lateral branch removed	39	10.4
Five needle fascicles removed	20	9.4
Nonwounded	5	15.8

^aBased on 75 seedlings per wound treatment.

age (two tissue ages \times three wound treatments \times 40 seedlings = 240 total seedlings). After inoculation, half of the seedlings in each treatment were moist-incubated for 24 hr by wetting the foliage and covering the seedling with a wet plastic bag. The other seedlings were placed in the greenhouse without further treatment.

Dieback of the stem distal to the inoculation point was recorded up to 4 mo after the inoculation treatments. Chi-square tests and analysis of variance were used to compare treatment differences.

RESULTS

1. Inoculum density. The four highest inoculum densities caused equally high amounts of dieback (Table 1). There was no effect of slit or pinhole wound treatment on infection, and the combined data are presented. No seedlings in the water control had dieback.

2. Age of wound. Age of wound and host species had a highly significant effect on dieback (Table 2). Fresh wounds were significantly more susceptible than older wounds. Loblolly seedlings were more susceptible to dieback than were slash seedlings. There was no effect of wound treatment, nor was the interaction of wound treatment by wound age significant.

3. Wound types. Pinholes and slit wounds were more effective as courts of entry for the pitch canker fungus than were wounds from lateral branch and needle removal (Table 3). Dieback also occurred more quickly in these treatments than in the other treatments.

4. Tissue age. One-year-old loblolly pine stem tissue was as susceptible (51% of 120 seedlings had dieback) as was succulent, current stem tissue (61%). Moist incubation for 24 hr after inoculation did not significantly increase dieback (61%) over the nonmoist treatment (51%).

DISCUSSION

In a loblolly pine seed orchard, dieback caused by *F. m.* var. *subglutinans* usually occurred near the tip of the second growth flush (14). Susceptibility to infection does not appear to be limited to the youngest tissue, according to the results of this study. In the field, fall and winter inoculations were more successful than those in spring and summer (1,14). Branches are more mature in fall and winter than in spring and summer, so factors other than tissue maturity affected susceptibility in the orchard.

Pinhole and knife-slit wounds have been used by others to demonstrate the susceptibility of southern pines to pitch canker disease (2,4,6,14,15). These wounds are equally effective as courts of entry for the pathogen. Needle removal simulating defoliation and a clipped lateral branch simulating cone harvest were not as effective. The infrequent infection of nonwounded seedlings supports Hepting and Roth's (12) contention that wounds are necessary for infection.

Because the fungus can invade 3- to 21-day-old wounds, a wounding agent such as an insect does not have to be the vector. The ready availability of spores in the air and on diseased tissue (4,9,14) suggests a vector is unnecessary.

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