

# New Method for Assessing Contamination of Slash and Loblolly Pine Seeds by *Fusarium moniliforme* var. *subglutinans*

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## ABSTRACT

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*Fusarium moniliforme* var. *subglutinans* (*F. m. subglutinans*), which causes pitch canker disease of pines in the southern United States, can be isolated from a pine seed by placing the seed on blue filter paper in a plastic tray, crushing it, and spraying it and the blotter paper with a liquid medium semiselective for *Fusarium* spp. The tray is covered and incubated at room temperature (about 20 C) for 14 days or until the colonies are 2 cm in diameter. Each colony is examined microscopically for the polyphialides diagnostic for *F. m. subglutinans*. This method permits rapid screening of representative samples of pine seed lots for *F. m. subglutinans* contamination and gives the same result as using a selective agar medium.

Pitch canker, caused by *Fusarium moniliforme* var. *subglutinans* Wollenw. & Reink. (*F. m. subglutinans*), is an economically important disease of planted slash and loblolly pines in the southern United States. Infections cause terminal and lateral branch dieback leading to stem deformity, reduced growth rate, and tree mortality (2). Other southern pine species are susceptible to pitch canker, but damage to these species usually is not economically significant (4).

In addition to dieback, this fungus can cause flower, cone, and seed losses in slash pine (5), and the infected seeds may serve as an inoculum source for damping-off and other diseases in nursery beds. Furthermore, seedlings grown from infected seeds may carry the pathogen to outplanting sites where additional losses may occur (3). To prevent such losses as well as exportation of the pathogen to areas where it does not exist, a method is needed for rapidly screening seed lots for *F. m. subglutinans* contamination.

Anderson et al (1) determined internal contamination in 21 slash pine seed lots by a laborious process of seedcoat disinfection, dissection, surface-sterilization of gametophyte and embryo tissues and culturing in a broad-spectrum medium. They reported 34% of seeds internally contaminated. The figure probably would have been far higher if external contamination had been included.

This method was not practical for

large-scale application. George Blakeslee of the University of Florida (*personal communication*) later refined the technique by placing pine seeds on an agar medium that was semiselective for *Fusarium* spp. and crushing them. This method was reliable, but it too was time consuming. Plant pathologists commonly assess fungal contamination of agricultural seeds by placing the seeds on blotter paper and supplying water. We modified this technique by using selective media and crushing the seed. Results on blotters were compared with those obtained on agar plates. This paper reports the successful use of the paper blotter method combined with selective media for assessment of *F. m. subglutinans* on and inside slash and loblolly pine seeds.

## MATERIALS AND METHODS

**Blotter method.** A broth was made by stirring 15 g of peptone, 5 g of MgSO<sub>4</sub> · 7H<sub>2</sub>O, 1 g of KH<sub>2</sub>PO<sub>4</sub>, and 1 g of 75% PCNB (Terraclor 75WP) into 1 L of distilled water, autoclaving 15 min at 100 kPa, cooling to room temperature, and adding 1 g of streptomycin sulfate and 0.12 g of neomycin sulfate. This broth was sprayed on a sheet of steel-blue blotter paper (Anchor Seed Germ Papers, St. Paul, MN) that had been placed in the bottom of a sterile, transparent, plastic box (133 × 133 × 32 mm) until saturated. Twenty-five single, unsterilized seeds were evenly placed on the blotter paper and crushed simultaneously by pressing a sterilized, heavy Plexiglas plate (cut to the dimensions of the box) against the seeds.

Sterile, transparent lids were placed on the boxes, which were incubated under ambient conditions (about 20 C, fluorescent light at irregular intervals and durations) until the colonies were about 2 cm in diameter (about 14 days). Each colony was examined under a light microscope at ×100–400 to determine if

the observed fungus was *F. m. subglutinans*. A colony was classified as *F. m. subglutinans* if characteristic microconidia, macroconidia, and polyphialides were present. Colonies with microconidia in chains, with pear-shaped microconidia, or with chlamydoconidia were not classified as *F. m. subglutinans* even though polyphialides were present.

Five slash pine and two loblolly seed lots were selected for evaluation by this method. Three hundred seeds were examined from each seed lot.

**Blotter vs. agar plate methods.** Three of the seed lots, with differing amounts of *F. m. subglutinans*, were assessed by an agar plate method as well as the blotter method. Four 25-seed replicates were assessed by each technique. The blotter plates were prepared as described. The agar plates were prepared using broth as for the blotter method but with agar added to 2% concentration and one crushed seed placed in the center of each agar plate. Seeds were incubated until the colonies were about 2 cm in diameter (about 14 days). All were examined under a light microscope to determine the incidence of *F. m. subglutinans*.

## RESULTS AND DISCUSSION

Between 0 and 68% (av. 24%) of seed lots examined from the five slash pine and two loblolly pine seed lots yielded *F. m. subglutinans* by the blotter method, whereas 2–63% yielded *F. m. subglutinans* by the agar method. Analysis of variance showed significant variation in occurrence of *F. m. subglutinans* by seed lot but not by detection method or interaction of method and seed lot. Frequency of

**Table 1.** Detection of *Fusarium moniliforme* var. *subglutinans* (*F. m. subglutinans*) associated with seeds of slash and loblolly pine using the PCNB agar plate method and the PCNB blotter paper method

Seed lot	Frequency of detection of <i>F. m. glutinans</i> by <sup>a</sup>					
	Agar method (replicates)			Blotter method (replicates)		
	1	2	3	1	2	3
1	5	5	4	3	5	5
2	47	48	45	46	44	43
3	13	19	12	14	18	16
4	59	63	56	60	52	68
5	20	12	18	12	20	15
6	22	18	12	24	20	20
7	2	4	2	4	0	8
Average	24	24	22	23	23	25

<sup>a</sup>Percentage of 100 seed (total of four groups of 25).

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detection of *F. m. subglutinans* on agar (22–24%) and on blotters (23–25%) was similar (Table 1).

The blotter-crushed seed method allows rapid assessment of 25 seeds at a time. The blue blotter paper provides good contrast for colony examination, and the selective medium retards growth of most non-*Fusarium* species. The seedcoats may shatter, especially with empty seed, but remain in the area of the crushed seed. The main task, therefore, is to separate *F. m. subglutinans* from other *Fusarium* species. This is made easier if the sample examined under the light microscope is picked from the seed instead of the blotter paper, where the colony is oldest and polyphialides are more common.

This new method is quick, reliable, and more cost-effective than the agar method. Twenty-five seeds can be screened quickly in one container. The agar method requires that agar be prepared, and about five seeds can be plated per dish, depending on plate size, thus requiring more supplies and time. By surface-sterilizing the seed and then following this procedure, the amount of internal *F. m. subglutinans* can probably be assessed, though this was not determined during the evaluation described in this report. This is important because the external fungi can be controlled effectively, but as yet, no methods are available to control internal fungi. Also, the method may be effective for other crops and other plant tissues.

#### LITERATURE CITED

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