MANAGEMENT OF DISEASE LOSSES THROUGH SEED TREATMENTS OF LONGLEAF AND SHORTLEAF PINES

L.D. Dwinell* and S.W. Fraedrich

The exclusion of fungal pathogens is an important strategy for managing disease problems in forest-tree nurseries. The pitch canker fungus, *Fusarium subglutinans* f. sp. *pini* (FSP), has been identified as a significant problem in many pine seed orchards and nurseries. The fungus causes strobilus mortality, seed deterioration, and cankers on the main stem, branches, and shoots of pines. The pitch canker fungus causes pre- and post-emergence damping-off and stem cankers on seedlings in southern pine nurseries. A source of inoculum for diseases in nurseries caused by the pitch canker fungus may be contaminated seeds. Significant losses of longleaf (*Pinus palustris*) and shortleaf (*P. echinata*) pine seedlings from FSP have been observed in state, federal, and private nurseries in several southern states. It may be possible to exclude the pitch canker fungus from forest-tree nurseries by seed treatments.

Shortleaf pine. We have studied the contamination of cones and seeds of shortleaf pine by the pitch canker fungus at a federal seed orchard in North Carolina. The isolation frequency of FSP from the surface of shortleaf pine seeds was a function of clone and sampling date. Varying the harvest date, however, did not reduce contamination to acceptable levels. The fungus was recovered from an average of 61% of the freshly extracted seeds. Only 1.6% of the shortleaf pine seeds were infested internally by FSP.

Since the contamination of the shortleaf pine seeds was largely on the seedcoat, it should be possible to eradicate the fungal pathogen by appropriate seed treatments. We found that the pitch canker fungus could be eliminated from the surface of shortleaf pine seeds in the laboratory by treating the seeds in 70 percent ethyl alcohol for 15 seconds, followed by a 60-second soak in 1% sodium hypochlorite. This treatment, however, may be impractical for decontaminating large volumes of seeds required by pine nurseries.

This past spring, in a pilot study at the Georgia Forestry Commission Flint River Nursery, we treated 3.63 kilograms of shortleaf pine seeds contaminated with FSP, and sowed them in nursery beds after stratification. The seeds were treated in 30% hydrogen peroxide for 15 minutes, followed by three water rinses. Although the final data has not been taken, there has not been any evidence of disease in the nursery seedlings.

Longleaf pine. Research on longleaf pine seeds indicate that FSP is primarily associated with the seedcoats, and infection of the endosperm and embryos are rare. In the laboratory, we have found that longleaf seeds can be decontaminated by treatment with a 30% hydrogen peroxide solution for 55 minutes.

We have studied the affects of various seed treatments on the germination of longleaf pine seeds and survival of longleaf pine seedlings. The research was conducted in containers (peat-based medium)

at the USDA Forest Service Ashe Nursery near Brooklyn, Mississippi, and in raised beds (sandy loam soil) at the Whitehall Experimental Forest in Athens, Georgia. At each location, seeds of 2 seedlots (Alabama and North Carolina) naturally-contaminated with FSP and other *Fusarium* spp. were treated with Arasan, a bacterium (*Burkholderia cepacia*), and hydrogen peroxide at 3% and 30% concentrations. Arasan (tetramethylthiuram disulfide) is a fungicide and animal repellent used as a seed treatment by many nurseries. The bacterium, *B. cepacia*, is being tested as a possible biological control agent. Hydrogen peroxide has been used by Barnett (1976. Tree Planters' Notes 27:17-24) as a means to enhance germination of southern pines. Laboratory tests have confirmed that hydrogen peroxide can be used to eradicate fungi on the surface of pine seeds. The treatments were untreated control, bacterium, 30% hydrogen peroxide (HP30), 3% hydrogen peroxide (HP3), Arasan, HP30 x bacterium, HP30 x Arasan, HP30 x Arasan x bacterium, and HP3 x Arasan. There were 96 seeds per replicate and 4 replicates per treatment. The experiment was established in the spring of 1997, and the number of live seedlings was determined after about two months. The data were analyzed by two-way analysis of variance. The Tukey's Studentized Range Test was used for means separation.

In Athens, the difference in the mean number of longleaf pine seedlings among the different seedlots was greater than would be expected after allowing for the effects of differences in treatments (Table 1). The mean number of seedlings for the Alabama and North Carolina seedlots was 49.4 and 26.4, respectively. The differences in the mean number of seedlings among the different treatments was not great enough to exclude the possibility that the differences were due to random sampling variability after allowing for the effects of differences in seedlots. The mean number of seedlings per treatment combination averaged 37.9 with a range of 34.2 to 41.4. There was no statistically significant interaction between seedlots and treatments.

At the Ashe Nursery, the differences in the mean number of longleaf pine seedlings among seedlots and treatments was greater than would be expected by chance. The mean number of seedlings for the Alabama and North Carolina seed sources was 34.2 and 18.6, respectively (Table 1). Treatments in which Arasan and the bacterium were used in combination with each other or HP30 had the lowest number of seedlings (Table 2).

Conclusions. The external contamination of longleaf and shortleaf pine seeds by fungal pathogens, including the pitch canker fungus, can be eradicated by appropriate seed treatments. Hydrogen peroxide shows promise as a seed disinfectant, but additional research is needed on concentration and exposure time. Furthermore, these results suggest that for containerized-longleaf pine, seed treatments may work best alone than in combinations. The combination of HP30 x Arasan, however, was the best treatment in a similar experiment in 1996 at the Ashe Nursery (unpublished data). The 1997 results may be confounded by poor seed quality. Seed treatments that exclude the pathogens from nursery soils may have long-term effects on the incidence of disease. This is a topic for future research.

Table 1. Differences in longleaf pine seedling production from 2 seed sources in seed treatment experiments at the Ashe Nursery (Mississippi) and Whitehall Forest (Georgia) in 1997.

Nursery location[a]	Seed source	Number of seedlings[b]
Ashe Nursery	Alabama	34.2 a
	North Carolina	18.5 b
Whitehall Forest	Alabama	49.4 a
	North Carolina	26.4 b

[a]The longleaf pine seeds were sown in containers at the Ashe Nursery and in raised beds at the Whitehall Forest.
[b]The number of seedlings is based on 96 seed per replication. Means within Nursery locations followed by the same letter are not significantly different according to Tukey’s Studentized Range Test.

Table 2. The effects of different seed treatments on longleaf pine seedling establishment in containers at the Ashe Nursery in 1997.

Treatments	Number of seedlings[a]
Control	33.2 ab
Bacterium (B)	30.0 abc
Hydrogen peroxide 30% (HP30)	25.4 abcde
Arasan (A)	29.4 abcd
B x A	19.6 cde
HP30 x B	23.1 bcde
HP30 x A	22.9 bcde
HP30 x A x B	16.8 e
Hydrogen peroxide 3% (HP3)	35.8 a
HP3 x A	27.8 abcd

[a] Data are means of 4 replicates (96 seeds were treated per replicate). Means followed by a common letter are not significantly different according to Tukey's Studentized Range Test.

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