STERILIZING SOUTHERN PINE SEEDS WITH HYDROGEN PEROXIDE

For best results, loblolly seeds should be soaked for 30 minutes to 1 hour, slash pine no longer than 1 hour, shortleaf no longer than 15 minutes, and longleaf about 1 hour in a 30-percent hydrogen peroxide solution.

James P. Barnett
principal silviculturist, Forest Service, U.S. Department of Agriculture, Southern Forest Experiment Station, Pineville, La.

Like most tree seeds, those of southern pine are infested with parasitic and saprophytic microorganisms (7, 8). Infestations can reduce seed vigor (10) and may infect the new crop during the vegetative period, particularly in greenhouses or nurseries, where disease epidemics develop rapidly. Seedcoats infested with fungi can also cause damping-off and root-rot diseases in containerized seedlings (6). Control of the microorganisms infesting conifer seeds may be accomplished by coating the seeds with a fungicide or by sterilizing the seedcoats. However, most of the fungicides evaluated for forestry have shown considerable phytotoxicity (3, 11), and many sterilants inhibit the germination of some species (5).

Hydrogen peroxide has successfully sterilized the seeds of several tree species (9); it has also been evaluated as a germination stimulant for both western conifers and southern pine seeds (1, 2, 4). Instead of stratifying loblolly (Pinus taeda L.) and slash (P. *elliottii* Engelm.) pine seeds, Carter and Jones (2) recommended soaking them in a 1-percent hydrogen peroxide solution. The present paper reports fungal development and germinability of seeds of four principal southern pine species after various hydrogen peroxide soaking treatments.

Methods

Sterilization treatments -Three separate seed lots each of slash, loblolly, longleaf (P. *palustris* Mill.), and shortleaf (P. *echinata* Mill.) pine were collected in central Louisiana in the fall of 1972 or 1971 and stored at 25° F. The lots were selected to provide a range in seed vigor. Each lot was divided to provide for three replications of nine treatments consisting of soaking the seeds in two hydrogen peroxide concentrations for various periods. Treatments were no soaking (control), soaking in a 3percent solution for 4, 8, 24, or 48 hours, and soaking in a 30-percent solution for 15 minutes, 30 minutes, 1 hour, or 3 hours. The treatment times were staggered so that all soaks were completed simultaneously for evaluations of sterility and viability. All soaking was done at room temperature (75° F).

Germination and sterility tests- After the soaking treatments, 100-seed samples of each lot were taken to test germinability under standard laboratory conditions. Sterility was evaluated by counting the percentage of seeds that showed fungal colonies within 3 days after duplicate 50-seed samples were placed on a sterilized maltyeast medium. Germination and sterility data were tested for statistical significance at the 0.05 level by analyses of variance and multiple-range tests.

Results

Sterility and germinability after soaking treatments varied by species and seed lots.

Lololly Pine

Ail of the soakings markedly reduced contamination on loblolly seeds (table 1). Viability of these seeds, which have a hard seedcoat, appeared less affected by the treatments than the other three species. The longest soak in 30-percent hydrogen peroxide greatly reduced germination, though the lot with the lowest viability was less affected than the others. Soaking for 30 minutes to 1 hour in a 30-percent solution should sterilize the seeds without reducing germination.

Slash Pine

Fungal infestations of slash pine seed were completely controlled by all soaking periods in a 30percent solution (table 2). None of the soakings in the 3-percent solution were satisfactory, as they reduced infestations by only 8 to 17 percentage points.

Slash pine seeds did not germinate as well after soaking as loblolly. The longest soakings in both concentrations reduced germination, but seed lots of low initial viability appeared slightly improved by all but the longest soakings. Slash pine seeds should therefore be soaked for no longer than 1 hour in a 30-percent solution.

Shortleaf Pine

A// soaking periods of both concentrations completely surface-sterilized shortleaf seeds (table 3). However, the infestation of the untreated controls averaged only 15 percent. These levels, which were much lower than those of the other tree seeds, may not be representative of shortleaf pine.

Germination trends of shortleaf were similar to those observed for slash pine in that lengthy soaks were usually detrimental to viability. Shortleaf should probably be soaked for no longer than 15 minutes in a 30-percent solution.

Longleaf Pine

All controls showed 100 percent infestation, but soaking for 30 minutes or more in the 30-percent concentration completely eliminated microorganisms (table 4). Results for the 3-percent solution varied extensively, though none were satisfactory. The development of microorganisms after the long soakings in the 3 percent solutions may have been due to an external fungal growth originating internally in contaminated nonviable seeds.

Longleaf seeds -particularly from lots with low-viability benefited from 30- to 60-minute soaks in the 30-percent solution. In one case, soaking seeds for 3 hours in a 30-percent solution increased germinability from 16 to 88 percent. The response appeared closely related to initial seed vigor. Longleaf should probably be soaked for about 1 hour in a 30-percent solution.

Table 1.—*Fungal infestation and germination of loblolly pine seeds soaked in hydrogen peroxide*

Treatment	Mean infestation[1]	Mean germination[1]
	Percent	
Control	99 (97–100)	91 (78–97)
3-percent solution		
4 hours	19 (1– 33)	87 (82–95)
8 hours	11 (6– 17)	93 (87–97)
24 hours	4 (0– 9)	93 (86–96)
48 hours	2 (0– 3)	94 (90–98)
30-percent solution		
15 minutes	0 (0– 1)	88 (85–90)
30 minutes	0	89 (84–96)
1 hour	0	90 (88–93)
3 hours	0	44 (15–93)

[1]Average of three seed lots. Range shown in parentheses.

Table 2.—*Fungal infestation and germination of slash pine seeds in hydrogen peroxide*

Treatment	Mean infestation[1]	Mean germination[1]
	Percent	
Control	54 (21–100)	81 (69– 99)
3-percent solution		
1 hour	43 (1– 84)	82 (73– 99)
8 hours	44 (2– 89)	79 (68–100)
24 hours	46 (11– 92)	50 (23– 97)
48 hours	37 (3– 84)	43 (18– 92)
30-percent solution		
15 minutes	0	83 (72–100)
30 minutes	0	85 (77– 98)
1 hour	0	84 (76– 98)
3 hours	0	75 (70– 82)

[1]Average of three seed lots. Range shown in parentheses.

Discussion

Although the optimum lengths and concentrations of hydrogen peroxide soakings varied by species and by the vigor of seed lots, soaking for 1 hour or less in a 30-percent solution appeared

Table 3.—*Fungal infestation and germination of shortleaf pine seeds soaked in hydrogen peroxide*

Treatment	Mean infestation[1]	Mean germination[1]
	Percent	
Control	15 (5—26)	76 (54—92)
3-percent solution		
4 hours	0	82 (66—92)
8 hours	0	80 (68—88)
24 hours	0	67 (50—80)
48 hours	0	73 (69—82)
30-percent solution		
15 minutes	0	82 (66—90)
30 minutes	0	75 (68—81)
1 hour	0	48 (17—85)
3 hours	0	7 (0—18)

[1]Average of three seed lots. Range shown in parentheses.

Table 4.—*Fungal infestation and germination of longleaf pine seeds soaked in hydrogen peroxide*

Treatment	Mean infestation[1]	Mean germination[1]
	Percent	
Control	100	53 (16—77)
3-percent solution		
4 hours	13 (5— 22)	36 (15—50)
8 hours	19 (3— 43)	26 (8—36)
24 hours	84 (75—100)	27 (24—34)
48 hours	82 (53—100)	3 (0— 4)
30-percent solution		
15 minutes	4 (2— 7)	49 (16—66)
30 minutes	0	63 (24—86)
1 hour	0	77 (66—80)
3 hours	0	54 (23—88)

[1]Average of three seed lots. Range shown in parentheses.

consistently most effective for all species. Soaks in the 3-percent solution-which is inexpensive and easily available commercially-greatly reduced the incidence of microorganisms; however, only complete control is thought acceptable because even small infected areas can quickly expand in greenhouses and cause heavy mortality. Therefore, it is best to apply the heavier concentration despite its greater cost.

The effects of the treatments on germinability varied greatly among species and among seed lots within species. Seed lots of low viability apparently benefit greatly from soaking, though germinability of highly viable seeds may decrease.

Literature Cited

1. Barnett. J P. and B F. McLemore
1967. Germination of loblolly pine seed hastened by soakings in aerated cold water. Tree Planters' Notes 18(2):24-25
2. Carter, M. C. and LeRoy Jones
1962. The effect of hydrogen peroxide on the germination of loblolly and dash pine seed. USDA For. Serv., Southeast. For. Exp. Stn.,Pap. 141. 12 p.
3. Cayford, J. H. and R. M. Waldron
1967. Effects of captan on the germination of white spruce, jack and red pine seed. For. Chron. 41:381.384
4. Ching, T. M. and M. C Parker
1958. Hydrogen peroxide for rapid viability tests of some coniferous tree seeds. For. Sci. 4:128-134
5. Neal, J. L., Jr., J. M. Trappe, K. C. Lu, and W. B. Bollen.
1967. Sterilization of red alder seedcoats with hydrogen peroxide. For. Sci. 13:104-105

(Continued on p. 24)

Continued From p. 19

6. Pawuk, W. H and J P Barnett
1974. Root rot and damping-off of container-grown southern pine seedlings Proc. North Amer Containerized For. Tree Seedling Symp., Great Plains Agric. Council 68:173-176

7. Schubert, G. H
1960. Fungi associated with viability losses of sugar pine seed during cold storage Proc. Soc Amer. Foresters 1960' 18-21

8. Shea, K R
1960. Mold fungi on forest tree seed. Weyerhaeuser Company For. Res. Note 31, 10 p.

9 Trappe, J. M.
1%1 Strong hydrogen peroxide for sterilizing coats of tree seed and stimulating germination J. For. 59:828-829

10. Urosevic, B
1961 The influence of saprophytic and semi-parasitic fungi on the germination of Norway spruce and Scots pine seeds. Proc. Int. Seed Test. Assoc. 26:537-556

11. Vaartaja, 0
1956. Screening fungicides for controlling damping-off of tree seedlings. Phytopathol 46 387-390

Continued From p. 21

Literature Cited

1. Grisez, T J.. and H I Huntzinger
1965. Direct seeding studies with black cherry. In Direct seeding in the Northeast - a symposium, p 41.43 Amherst, Mass

2 Huntzinger, H. J.
1968. Methods for handling black cherry seed USDA For. Serv Res Pap NF-102. 22 p , illus

3 Huntzinger, H. J
1971 Long-term storage of black cherry seed - is it effective· Tree Planters' Notes 22(4):3-4