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A Review of Chemical Treatments to Improve Germination of

Longleaf Pine Seeds

Photo by John McGilvray

ABSTRACT

Nursery managers can improve germination of longleaf pine (*Pinus palustris* P. Mill. [Pinaceae]) seeds and seedling establishment by reducing seedborne pathogenic fungi with appropriate sterilants and fungicides. We have tested many chemicals, but hydrogen peroxide, thiophanate methyl, and thiram seem to provide the best results in reducing the large populations of microorganisms carried on the large, thin, and fibrous seed coats. Our evaluations of longleaf seeds indicate that *Fusarium* spp. are major seedborne pathogens that cause mortality to seeds and newly germinated seedlings. Seeds of other southern pines have denser coats and are less adversely affected by the presence of seedborne pathogens. The increased demand for longleaf pine seeds in the last few years makes reducing this contamination an important consideration by seed dealers and nursery managers.

KEY WORDS
Pinus palustris, seed quality, seed coat pathogens

NOMENCLATURE
USDA NRCS (2002)

Interest in restoring longleaf pine (*Pinus palustris* P. Mill. [Pinaceae]) on many sites in the southern US has increased dramatically over the last decade. Although the quantity of seeds collected and seedlings produced has increased markedly, the quantity and quality of longleaf pine seeds remain insufficient to meet demand across the range of the species. Most nursery managers would like to be able to maintain 85% or higher germination and 90% or higher survival after seedling emergence. Otherwise, sowing more than 1 seed per container cavity will be necessary and the subsequent waste of seeds will jeopardize efforts to produce high-quality crops consistently and economically (Figure 1).

Seeds of other major southern pines (Pinaceae), loblolly (*P. taeda* L.), slash (*P. elliottii* Engelm.), and shortleaf (*P. echinata* P. Mill.) are relatively easy to collect, process, and maintain. So why are seed dealers and nursery managers unable to produce similar quantities of quality longleaf pine seeds? The answer to this question is related to the uniqueness of the species.

The relation between cone maturity and storage in longleaf is unique among southern pine species. Germination of longleaf seeds will not improve with storage if the cones are not ripe enough to be opened operationally (McLemore 1975). To ensure that seeds have high viability, cone collection must be delayed until the seeds are fully mature. This shortens the effective cone collection season.

Longleaf pines have a lower ratio of seed coat weight to total seed weight than other southern pines (Barnett 1976a; Carpita and others 1983). (This may explain why seeds of longleaf pine have little dormancy compared to the other species.) Longleaf pine seeds also have permanently attached wings, thin seed coats, and are unusually moist when extracted from cones. These traits result in longleaf seeds being the most difficult of the southern pines to successfully collect, process, store, and treat without adversely affecting quality (Wakeley 1954; Barnett and Pesacreta 1993). The fibrous, irregular surface of the seed coats provides a good foothold for microorganisms. Many of the fungi residing on seeds are pathogenic and cause seed and seedling mortality (Pawuk and Barnett 1974; Pawuk 1978), particularly in longleaf pine seeds of low vigor. Reducing infestations on seeds with chemicals has proved to be an effective means of improving longleaf pine seed health and performance (Barnett 1976b; Barnett and others 1999). We review early and recent efforts to determine efficacy of chemical treatments to improve longleaf seed germination and seedling establishment and relate these responses to those for other southern pines.

EARLY EVALUATIONS

For many years, the presence of fungi on southern pine seeds was not considered a problem because observations indicated the fungi were saprophytic and did not reduce germination (Belcher and Waldrip 1972). With the advent of container seedling culture it became apparent that seedborne fungi could be an important cause of seedling mortality. Pawuk and Barnett (1974) associated *Fusarium* infection of container longleaf pine seedlings with retention of infested seed coats. Testing was needed, then, to determine if reduction of microorganisms on longleaf seed coats would improve germination and reduce seedling mortality.

Sterilants

Hydrogen peroxide has been used successfully to sterilize seeds of several tree species (Trappe 1961); it also has been evaluated as a germination stimulant for western conifers and southern pine seeds (Ching and Parker 1958; Carter and Jones

Photo by James P Barnett

Figure 1. A healthy crop of longleaf pine seedlings growing in a southern nursery. Ideally, nursery managers sow a single seed per container cavity.

1962). So, disinfecting southern pine seeds with 3% and 30% hydrogen peroxide soaks was tested to determine their effects on reducing fungal contamination and improving germination (Table 1). The results indicated that longleaf pine seeds were heavily infested with fungi, but that a 1-h soak in 30% hydrogen peroxide effectively removed fungal contamination and also improved germination of low-vigor lots (Barnett 1976b).

Although the 1-h soak in 30% hydrogen peroxide has been used operationally to improve performance of longleaf pine seeds (Barnett and McGilvray 1997), it is a strong oxidant that can cause skin and eye injuries. Many organizations hesitate to use this treatment on a large scale because of safety concerns and have, therefore, looked to fungicidal seed treatments as a means to reduce fungal contamination.

Fungicides

Fungicides applied as seed coatings are commonly used in agriculture to reduce infestation of fungi on seeds, but the effect on pine seed germination was initially unknown. Several fungicides have been tested for their effect on the germination of longleaf, slash, loblolly, and shortleaf pine seeds (Pawuk 1978). Captan (Captan 50WP, Micro Flo, Lakeland, Florida; 50% active ingredient (ai): N-trichlorome-thylthio-4-cyclohexane-1,2-dicarboimide) and thiram (Thiram 42S®, Gustafson, Plano, Texas; 42% ai: tetramethylthiuran disulfide) had the least detrimental effect on germination—all other fungicides reduced germination of one or more of the 4 southern pine species (Pawuk 1978). This does not mean that some of these fungicides may not be effective in reducing fungal infestations. For instance, benomyl (Benlate 50WP®, Dupont, Wilmington, Delaware; 50% ai: methyl-(butylcarba-moyl)-2-benzimidazolecarbamate) has been an effective fungicide for seed and seedling application. A 10-min soak in a 2.5% ai benomyl solution was equally as effective as a 1-h soak in 30% hydrogen peroxide in controlling pathogens and improving germination of longleaf seeds (Barnett and McGilvray 1997; Littke and others 1997; Barnett and others 1999). However, benomyl has been withdrawn from the marketplace and other alternatives are needed.

RECENT STUDIES

Several compounds have been formulated as potential replacements for benomyl. Some are in the same chemical family and should have similar effectiveness as fungicides for seed and seedling application. Unfortunately, only limited evaluations of these, and other potential chemicals for use with longleaf pine, have been completed.

In a study by Barnett and McGilvray (2002), untreated and hydrogen peroxide-treated seeds were germinated and grown for 5 mo in small containers. During the growing period, seedlings received either no fungicide or biweekly applications of benomyl, thiophanate-methyl (Fungo 50WSB Flo®, Scotts-Sierra, Marysville, Ohio; 46.2% ai: dimethyl 4,4-0-phenyl-

TABLE 1

Germination and fungal infestation of southern pine seeds treated with hydrogen peroxide.

Hydrogen peroxide treatment	Loblolly Germination	Loblolly Infestation	Slash Germination	Slash Infestation	Shortleaf Germination	Shortleaf Infestation	Longleaf Germination	Longleaf Infestation
	%							
None	91	99	81	54	76	15	53	100
3%								
4 h	87	19	82	43	82	0	36	13
8 h	93	11	79	44	80	0	26	19
24 h	93	4	50	46	67	0	27	84
48 h	84	2	43	37	73	0	3	82
30%								
15 min	88	0	83	0	82	0	49	0
30 min	89	0	85	0	75	0	63	0
1 h	90	0	84	0	48	0	77	0
3 h	44	0	75	0	7	0	54	0

Source: Barnett (1976b).

TABLE 2

Longleaf pine seedling percentages from seed and seedling treatments.

Seedling treatment	Seed treatment [a]		
	Control	Hydrogen peroxide	Average
	%		
Control	78	92	85 a
Benlate®	85	93	89 b
Fungo-flo®	88	94	91 b
Fungo-flo® plus Subdue®	90	94	92 b
Average	85 a	93 b	

[a] Seedling percentages (numbers plantable in November divided by numbers with an initial germinant) are averages of 288 seedling cavities for each of 3 replications. Averages within columns and across rows followed by the same letter are not significantly different at the 0.05 level.

Source: Barnett and McGilvray (2002).

TABLE 3

Longleaf pine seed germination and fungal infestation, and seedling development following applications of several compounds to control seedborne pathogens. [a]

Seed treatments	Germination	Infestation	Seedling dry weight Shoot	Seedling dry weight Root
	%	%	g	g
Control	29 a	99 a	1.98 a	1.35 a
Hydrogen peroxide	43 c	3 c	2.02 a	1.03 c
Cleary 3336F ®	32 b	100 a	2.02 a	1.17 b
Pathguard 6F ®	32 b	84 b	2.61 c	1.29 ab
ZeroTol ®	32 b	100 a	2.29 b	1.24 b

[a] Means within columns followed by the same letter are not significantly different at the 0.05 level.

This study was a randomized experiment with 3 treatment replications of 150 seeds (6 trays of 50 seeds each) for germination testing, 3 replications of 45 seeds (9 dishes of 5 seeds each) for pathological evaluations of seed microorganisms, and 3 replications of 3 containers each (45 seedling cavities per container) for seedling establishment at 90 d.

Source: Barnett (2003).

enebis[3-thioallophanate]), or thiophanate methyl plus metalaxyl (Subdue 2E®, Novartis, Greensboro, North Carolina; N-(2,6-dimethylphenyl)-N-(methoxacetyl)-alamine methyl) (Table 2). This study showed that treating seeds with hydrogen peroxide improved seedling production without the need for fungicidal applications to seedlings during their growth period. The results also indicated that, in terms of improving seedling production, treating seedlings with thiophanate-methyl compounds is as effective as with benomyl.

In another study, thiophanate-methyl and chlorothanonil compounds were evaluated for their effect on seed germination and pathology and early seedling development (Barnett 2003). The treatments were: 1) an untreated control; 2) a 1-h soak in 30% hydrogen peroxide; 3) a 10-min soak in a 1.3 ml/l aqueous solution of thiophanate-methyl (Cleary 3336F®, Cleary, Dayton, New Jersey; 41.6% ai: dimethyl 4,4-0-phenylene-bis[3-thioallophanate]); 4) a 10-min soak in a 2 ml/l aqueous solution of chlorothanonil (Pathguard 6F®, Whitmire Micro-Gen, St Louis, Missouri; 54% ai: tetrachloroisophathalonitrile); and 5) a 10-min soak in a 1:50 dilution of hydrogen dioxide (ZeroTol®, BioSafe Systems, Glastonbury, Connecticut; 27% ai).

The 1-h soak in 30% hydrogen peroxide was the most effective treatment (Table 3). Chlorothanonil reduced seed infestations but to a more limited extent. Chlorothanonil also resulted in larger seedling top weights 90 d after germination. Although none of these chemicals were as effective as hydrogen peroxide, some may be more effective for longleaf pine seeds at higher application rates, or for other conifer species at recommended rates. Additional studies are needed to more thoroughly evaluate the efficacy of these treatments.

Since thiram (applied as a slurry at 5 ml/0.45 kg) has long been a labeled seed fungicide, it and some newer chemicals (Vitavax PC® at 2.5 ml/0.45 kg [8 oz/100 lb], Gustafson, Plano, Texas; 45% Captan; 15% PCNB® [pentachloronitrobenzene]; 10% Carboxin® [5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide], and ABG-3035™ [an unregistered fungicide] at 1 ppm [Gustafson, Plano, Texas]) were evaluated in combination for their potential in reducing seed coat fungal contamination and improving germination of southern pine seeds (Barnett and Varela 2003). Gustafson Seed Technology Center (McKinney, Texas) treated seeds and conducted both pathological and germination evaluations. Germination of samples also was conducted at the Pineville Seed Testing Facility. Results from the 2 facilities were similar, so only the Gustafson results are shown (Table 4).

The tests with longleaf pine showed that *Fusarium* spp. infested 32% of the seeds (Table 4). The thiram treatment reduced the number infested to 12% and increased germination from 53% to 72%. Although the combination of thiram and Vitavax further reduced infestation of the seeds to 2%, it did not significantly improve germination over the single thiram treatment.

TABLE 4

Levels of seed coat contamination and germination of longleaf pine seeds following treatment with fungicides.[a]

Seed treatments	Fungus infestation				
	Penicillium	*Colletotrichum*	*Fusarium*	*Rhizopus*	Germination
	%				
Control	58	2	32	10	53
Thiram 42S® with colorant	2	0	12	0	72
Thiram 42S®, ABG-3035™, with colorant	4	0	10	0	69
Thiram 42S®, Vitavax PC®	0	0	2	0	76
Thiram 42S®, ABG-3035™ Vitavax PC®	0	0	8	0	59
Vitavax PC®	0	0	16	0	70

[a] Testing was conducted at the Gustafson Seed Technology Center. Two replications of 50 seeds each were used for all treatment evaluations.
Source: Barnett and Varela (2003).

DISCUSSION

Longleaf pine seeds can carry a high infestation of microorganisms. *Fusarium* species seem to be the primary fungi that are pathogenic to seed germination and cause seedling mortality. Several studies have shown that treating seeds to reduce fungal contamination significantly improved seed health and increased both seed germination and seedling development.

Removing seed coat pathogens by use of a 1-h soak in 30% hydrogen peroxide not only improved seed germination but also reduced the need for fungicidal applications during seedling culture. Fungicides that seem particularly effective in improving seed performance include thiophanate methyl (a replacement for benomyl) and thiram. Other fungicides may have potential but initial testing has not demonstrated their effectiveness. These results show the significant benefit of using seed treatments on longleaf pine seeds to control fungi and improve germination. Chemicals effective for longleaf pine should work well with any other southern pine species.

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