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Short communication

Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed

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Abstract

Longleaf pine production in the southeastern United States can be limited by species of *Fusarium*, which decrease seed germination and seedling survival. Benomyl, difenoconazole, hydrogen dioxide, mancozeb, and thiabendazole were evaluated for their ability to inhibit the growth of four species of *Fusarium* and to enhance longleaf pine seed germination. Species of *Fusarium* did not grow on benomyl- or mancozeb-amended media. Only *F. solani* grew on thiabendazole and hydrogen dioxide-amended media. In laboratory studies, mancozeb and benomyl seed treatments significantly improved longleaf pine seed germination compared to controls. In greenhouse studies, fungicide treatment did not increase germination over nontreated controls. Recovery of *Fusarium* spp. from mancozeb and hydrogen dioxide treated seed was significantly lower than nontreated controls.

Keywords: *Pinus palustris*; Seed treatment; Systemic fungicides

1. Introduction

Species of *Fusarium* are responsible for a variety of conifer seedling diseases including seed rots, pre- and post-emergence damping-off, root rot, and late season damping-off (Carey and Kelley, 1994; Pawuk, 1978). While several *Fusarium* spp. may be involved, diseases caused by *F. circinatum* such as damping-off and late-season seedling blight are common in longleaf pine nurseries in the southeastern United States (Carey and Kelley, 1994; Pawuk, 1978). Seed-borne contamination is believed to be the primary source of *F. circinatum* (Fraedrich and Dwinell, 1996), which is brought into nurseries on seed from pitch canker infected orchard trees (Carey et al., 2001).

Contamination of longleaf pine (*Pinus palustris* Mill.) seed by *Fusarium* spp. was first documented by Pawuk (1978) who reported that several seed lots contained up to 20% infested seed including *F. moniliforme* (=*F. circinatum*). Anderson et al. (1984) also reported the presence of *F. circinatum* inside the seed coat of slash pine (*P. elliottii* var. *elliottii* Engelm.). Within the past 12 years, pitch canker and other diseases caused by *F. circinatum* contaminated seed have been found in other parts of the world including South Africa (Viljoen et al., 1994).

Several fungicides have been evaluated for efficacy as southern pine seed treatments including using benomyl to enhance germination (Barnett et al., 1999), thiram and a thiabendazole-DMSO mixture to control damping-off (Runion and Bruck, 1988; Runion et al., 1991) and hydrogen peroxide as a seed disinfectant (Barnett, 1976). However, an efficacious fungicide is not currently labeled to control seedborne *Fusarium* diseases in longleaf pine. The objectives of this research were to evaluate fungicides against four species of *Fusarium* commonly associated with longleaf pine seed and test their effects on germination of longleaf pine seed germination.

2. Materials and methods

2.1. Laboratory analysis of fungal inhibition

Fusarium isolates were: *F. circinatum* isolated from longleaf pine seed, *F. oxysporum* from white pine

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(*P. strobus L.*) roots, and *F. proliferatum* and *F. solani* from longleaf pine seedlings. All were stored on carnation leaf agar (CLA, 1.5% water agar and 5–7 carnation leaves per plate) at 4°C. Each isolate was transferred to malt yeast extract agar (MYE) containing fungicides (base rate): benomyl (Benlate 50WP, DuPont, Wilmington, DE, 50% a.i.—0.37 g), difenoconazole (Dividend 0.31 FS, Syngenta, Greensboro, NC, 32.8% a.i.—0.66 ml), mancozeb (Manzate 200DF, DuPont, 75% a.i.—0.40 g), and thiabendazole (Mertect 340F, Merck, Rahway, NJ, 42.3% a.i.—0.0133 ml), or hydrogen dioxide (Zero-Tolerance, BioSafe Systems, Glastonbury, CT, 23% a.i.—2.0 ml). Except for benomyl, which had a special use label for longleaf pine seed, the base rates were estimated from labeled rates for other crops. The recommended base rate for hydrogen dioxide was a 1:50 dilution.

Each fungicide, at 0, 1, 2, 3, 4, or 5 times the base rate was incorporated into MYE after autoclaving. An 8 mm plug from the leading edge of a 7–10-day-old culture of each species was inverted onto MYE in 9-cm petri dishes and incubated in the dark at 27°C. Each treatment was replicated five times. Colony diameters were measured after 14 days. The original 8 mm plug was inverted and transferred to MYE without fungicide and radial growth measured after 14 days. This study was conducted three times.

2.2. Laboratory assessment of seed germination

Seed were obtained from a 1996 collection from Okaloosa County, Florida (from International Forest Company, Odenville, AL). Fungicides (rate) were prepared by mixing benomyl (0.150 g), difenoconazole (0.100 ml), or thiabendazole (0.0008 ml) with 50 ml of sterile distilled water (SDW) and mancozeb (0.120 g) with 5 ml SDW. Benomyl, difenoconazole, mancozeb, and thiabendazole were applied to 60 g longleaf pine seed at 0, 0.5, 1, and 1.5 times the base rate and hydrogen dioxide (2.0 ml) was applied at 0, 1, 2, and 3 times the base rate. Sixty g of seed were soaked in fungicide suspensions or hydrogen dioxide for 10 min, then air-dried for 1 h in a laminar flow hood. Forty seed of each treatment were placed in covered plastic boxes (19 cm × 13 cm × 10 cm, Pioneer Plastics, Inc., Dixon, KY) on the surface of germination paper (2 layers, KIMPAK, National Packaging Services Corp., Green Bay, WI) moistened with 50 ml of SDW and kept at 21°C and 12 h photoperiods for 4 weeks on laboratory benches in a completely randomized design. Seed treatments were replicated 6 times and 8 times for fungicides and hydrogen dioxide, respectively, along with an SDW control. Germinated seed were counted after 4 weeks. The experiment was conducted twice.

2.3. Greenhouse assessment of seed germination

Seed used in this study were from a 1998 collection from the North Carolina Division of Forestry's Bladen Lakes Seed Orchard, Bladen Lakes State Forest, North Carolina. Fungicidal seed treatments were the same as those described above. Forty seed of each treatment were sown singly in 4 cm diameter × 9 cm deep plastic cells (Stuewe & Sons, Inc., Corvallis, OR) containing 85 cm^3 of ProMix (Premiere Horticulture, Inc., Red Hill, PA). Seed were placed on the surface of the ProMix and covered with sand. Containers were maintained in the greenhouse at 28°C / 20°C (day/night) for 6 weeks in a completely randomized design with three replications. Seedling emergence, was recorded weekly for 6 weeks. The study was repeated twice.

2.4. Fusarium recovery from treated seed

Seed from the Bladen Lakes seed orchard were treated as above. The methods used were those described by Anderson (1986) except that neomycin sulfate was excluded from the nutrient broth. Two pieces of sterile, blue blotter paper (Packaging Converters, Hudson, WI) were placed in 13.3 cm × 3.7 cm deep plastic boxes (Pioneer Plastics) and moistened with 19 ml of broth. Sixteen seeds were placed on the paper surface and their seed coats broken using a sterile plexiglass plate. One-hundred microliters of broth was pipetted over each seed and boxes were incubated at 21°C and 12 h UV-light/12 h dark. After 7 days, colonies of *Fusarium* spp. were counted. Random colonies were transferred to CLA for identification (Nelson et al., 1983; O'Donnell et al., 1998). The experiment was conducted three times.

2.5. Data analysis

Data were analyzed using the PROC GLM procedure in SAS Version 8.0 (SAS Institute, Cary, NC). The means from each experiment were separated by Fisher's least significant difference (LSD) test ($P = 0.05$).

3. Results

3.1. Laboratory analysis of fungal inhibition

Growth of each of the species of *Fusarium* was completely inhibited on MYE amended with benomyl or mancozeb, regardless of fungicide concentration. Growth of *F. solani* was reduced by only 60% on difenoconazole-amended MYE regardless of concentration while all other species were reduced by 90% (data not shown). Media amended with thiabendazole completely inhibited growth of all species except *F. solani* regardless of concentration. Hydrogen dioxide in MYE

completely inhibited growth of the fungi, except for slight growth (7% of control) of *F. solani* at the lowest concentration. When fungal plugs were removed from fungicide-amended MYE, growth resumed from those plugs previously exposed to benomyl, thiabendazole, and difenoconazole but not from plugs exposed to mancozeb or hydrogen dioxide.

3.2. Laboratory assessment of seed germination

The germination rate of nontreated seed was 75% to 80% in laboratory trials. Seed treatment with benomyl or mancozeb increased seed germination compared to nontreated controls (Table 1). Seed treated with benomyl and mancozeb were significantly different from untreated controls. Seed treatment with thiabendazole, difenoconazole, or hydrogen dioxide did not significantly affect germination in laboratory trials.

3.3. Greenhouse assessment of seed germination

Seed treated with mancozeb at the 1.0× and 1.5× rate significantly increased percent germination over controls (Tables). All other treatments did not significantly differ from untreated controls.

3.4. Fusarium recovery from treated seed

Increasing rates of mancozeb and hydrogen dioxide (Table 1) resulted in lower *Fusarium* recovery compared to nontreated controls. The frequency of recovery of *Fusarium* spp. from seed treated with difenoconazole or thiabendazole did not significantly differ from the nontreated control.

4. Discussion

Seedling losses due to *Fusarium* spp. have been a problem throughout the longleaf pine nursery industry. Three systemic fungicides, a protectant fungicide, and a disinfectant were evaluated for their efficacy against species of *Fusarium* in vitro and on longleaf pine seed. In the current study, only benomyl showed some efficacy against *Fusarium* in longleaf pine seed. Mancozeb, a protectant, and hydrogen dioxide, a disinfectant, also exhibited some efficacy.

Two seed sources were used in these studies, a Florida source and a North Carolina source. Species of *Fusarium* were recovered from more than 90% of seed from North Carolina. Incidence of *Fusarium* infestation in the Florida seed was not determined. In the laboratory seed germination studies with Florida seed, nontreated seed had a high rate of germination and mancozeb and benomyl increased seedling production. In greenhouse studies, in which North Carolina seed were used, only mancozeb improved the germination rate. The differences in product performance between the laboratory and greenhouse studies may have been due to seed source.

Benomyl and thiabendazole improved germination of longleaf pine seed in previous studies (Barnett et al.,

Table 1
Percent laboratory germination four weeks after longleaf pine seed treatment, percent germination six wks after treatment, and percent *Fusarium* recovery 7 days after seed treatment

Treatment	% Laboratory germination[a]		% Greenhouse germination[b]		% Laboratory recovery[c]	
Control	81	ef	28	bcd	99	a
Benomyl 0.5×	86	abc	25	cd	99	a
Benomyl 1.0×	87	ab	30	bcd	84	b
Benomyl 1.5×	87	ab	39	ab	97	a
Difenoconazole 0.5×	81	def	23	d	97	a
Difenoconazole 1.0×	80	ef	24	d	97	a
Difenoconazole 1.5×	79	f	21	d	100	a
Mancozeb 0.5×	85	abcde	31	bcd	87	b
Mancozeb 1.0×	88	a	46	a	25	c
Mancozeb 1.5×	86	abcd	43	a	13	de
Thiabendazole 0.5×	84	abcde	24	d	99	a
Thiabendazole 1.0×	82	cdef	21	d	99	a
Thiabendazole 1.5×	81	def	25	cd	100	a
Hydrogen dioxide 1×	80	ef	36	abc	18	cd
Hydrogen dioxide 2×	78	f	30	bcd	7	e
Hydrogen dioxide 3×	83	bcdef	26	cd	6	e
LSD	5.08		4.58		1.22	

[a] Values are the combination of two repeated studies ($n=12$, and 16).
[b] Values are the combination of three repeated studies ($n=9$).
[c] Values are the combination of three repeated studies ($n=9$). Values within a column not followed by the same letter are significantly different ($P=0.05$) according to a test of Fisher's least significant difference.

1999; Runion and Bruck, 1988). In contrast, thiabendazole did not improve longleaf pine seed germination in the current study; however, seed treatment rates were lower than those used previously (Runion and Bruck, 1988). In our study, benomyl improved longleaf pine seed germination, completely inhibited growth of species of *Fusarium* in vitro, but did not improve seedling germination. In previous studies on longleaf pine seed, rates of benomyl were higher (Barnett et al., 1999) than those used in the current study. Benomyl has been removed from the US market by the EPA, so additional studies with benomyl are not warranted.

Difenoconazole is a systemic fungicide that has not previously been evaluated on longleaf pine against species of *Fusarium*. This fungicide has previously been shown to improve seed germination of wheat (Forster and Strausbaugh, 1998) and corn (Munkvold and O'Mara, 2002). In the current study, difenoconazole had limited efficacy against four *Fusarium* species in vitro, and did not improve longleaf pine seed germination.

Mancozeb has provided control of *Fusarium* spp. on seeds, tubers and seedlings of a variety of agricultural crops (Agrios, 1997). In the current study, mancozeb inhibited growth of four species of *Fusarium* in vitro, increased longleaf pine seed germination in laboratory studies, and reduced recovery of species of *Fusarium* from longleaf seed. Our results indicate a need for further evaluations of this fungicide in nursery settings.

Hydrogen dioxide is labeled as a disinfectant to reduce fungal and insect pests in ornamental nurseries. Specifically, incidence of *Phomopsis vaccinii* in blueberries (Schilder et al., 2002) was reduced with applications of hydrogen dioxide. In the current study, hydrogen dioxide treatment did not affect longleaf pine seed germination but did inhibit *Fusarium* growth in vitro. Given that no phytotoxicity was observed in seed germination trials and that recovery of *Fusarium* was reduced with hydrogen dioxide, further trials might be desirable regarding the use of this product on longleaf pine.

In the pine nursery industry, seedlings are sold in quantity, and consistent germination is critical. Since sources of high quality longleaf pine seed are not readily available, most notably because of poor seed germinability (Barnett, 1999) due to seedborne *Fusarium*, methods to increase seed germination are important. Fungicidal seed treatment is an inexpensive means of disease control that can help prevent seedling losses from a variety of fungal pathogens (Agrios, 1997). While these treatments could increase the overall number of marketable seedlings, concerns exist regarding fungicide use on longleaf pine seed and the possibility of phytotoxicity, particularly with systemic fungicides or high rates of application. The current study confirms existing products can improve germination of *Fusarium*-infested longleaf seed without phytotoxicity.

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