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Cooperative

RESEARCH REPORT 00-1

INHIBITION OF FOUR *FUSARIUM* SPECIES ASSOCIATED WITH LONGLEAF PINE AND THEIR EFFECTS ON SEED GERMINATION

by
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INTRODUCTION

Some of the most important plant pathogens, responsible for wilts, blights, root rots, and cankers across a wide variety of agricultural crops and trees worldwide, belong to the genus *Fusarium*. *Fusarium* spp., in general, are considered facultative parasites, having the ability to survive on nutrients with or without a living host. Twenty-seven of the 43 recognized species are considered pathogenic on green plants and some of the same *Fusarium* sp. pathogenic to agricultural crops are found associated with longleaf pine (*Pinus palustris* Mill.) seed. These include *Fusarium oxysporum, F. proliferatum, F. solani,* and most importantly, *F. circinatum* (= *F. subglutinans* f. sp. *pini*) which is capable of infecting all life stages of southern pines.

Fusarium circinatum causes damping-off and late-season seedling blight of longleaf pine in both containerized and bareroot nurseries. Late-season seedling blight is characterized by a sudden and rapid death of seedlings which occurs during the first growing season. The early symptoms of late-season seedling blight first appear as water-soaked tissues on the bud interior, which begins to darken as necrosis progresses. Seed-borne contamination is believed to be the primary source of *F. circinatum* inoculum for damping-off and late-season seedling blight seedling diseases in longleaf nurseries. Some researchers suggest that *F. circinatum* is carried into nurseries on or in seed which originate from pitch canker-infected trees in seed orchards. However, while studies have yet to find a strong correlation between infected trees in the field and infested seed in the nursery, the disease distribution in the nursery is characteristic of seed transmission.

The forest tree nursery industry is in dire need of an effective seed treatment for improved production of longleaf pine seedlings. The overall objective of this research was to determine the inhibitory effects of labeled fungicides on the *in vitro* mycelial growth, of *Fusarium* sp. commonly associated with longleaf pine seed. Two hypothesis were tested: 1) *in vitro* analysis of fungicides for their inhibitory effect on mycelial growth will identify potential treatments for the control of seedborne *Fusarium spp.*, and 2) chemicals applied to longleaf pine seed will not inhibit germination and may identify an acceptable seed treatment for the control of *Fusarium* spp.

METHODOLOGY

In Vitro agar analysis - Hypothesis 1

Fungal species

The four *Fusarium* species used for experiments were recovered from infected southern pines and were identified as *Fusarium circinatum, F. oxysporum, F. proliferatum* and *F. solani*. All four have been shown to be associated with longleaf pine seed.

Chemicals

The label rate of each fungicide tested was converted to add into 100 mL of malt yeast extract agar (MYE) and treat 60 g of longleaf seed. *In vitro* agar experiments included five chemical rates, 1x, 2x, 3x, 4x, 5x, and a control (Table 1).

Table 1. Fungicide used to amend malt yeast extract agar plates. The 1x rate for each fungicide was converted for a 30 g sample of longleaf pine seed and into 100 mL of molten agar.

Trade name	Common name	Producer	Action	1xRate/100mL
Agribrom®	----------	Great Lakes Chemical	Microbial biocide (disenfectant)	0.16 g
Apron® 30 FS	Metalaxyl	Novartis	Fungicide seed dressing	0.13 ml
Baytan®	Triadimenol	DuPont	Systemic fungicide Seed Treatment	0.20 ml
Benlate®	Benomyl	DuPont	Systemic fungicide	0.37 g
Dividend®	Difenoconazole	Novartis	Systemic fungicide	0.66 ml
Heritage®	Azoxystrobin	Zeneca	Fungicide	0.02 g
Manzate®200DF	Mancozeb	DuPont	Seed treatment	0.40 g
Mertect® 340F	Thiabendazole	Merck	Systemic fungicide	0.0133 ml
Premis®	Triticonazole	Rhone-Poulenc	European fungicide label	0.20 ml
Tilt®	Propiconazole	Novartis	Foliar fungicide Seed Treatment	0.007 ml
Zero-Tolerance®	----------	BioSafe	Microbial biocide (disenfectant)	2.0 ml

In vitro analysis

A 4 mm agar plug was removed from the leading edge of a 7-10 day old *Fusarium* culture and placed on each chemically-amended MYE plate. Radial growth of each *Fusarium* sp. was recorded at 3, 5, 7, and 14 days for all chemical rates. To determine whether the chemical was fungistatic or fungicidal agar plugs were removed at 14 days from amended media, inverted, and placed on a non-amended MYE plate. Non-amended plates were placed in an incubator and radial growth measured as described above. Experiments were repeated twice for a total of three experiments.

In Vitro seed germination analysis - Hypothesis 2

Seed source

Longleaf pine seed with a 92% germination rate was obtained from International Forest Company (Odenville, AL) and used throughout the duration of the study.

Chemicals

Chemical treatment of longleaf pine seed included 0.5x, 1x, and 1.5x label rates for Benlate®, Dividend®, Manzate®, and Mertect®, and 1x, 2x, and 3x for Zero-Tolerance® (Table 2) and were based on 60 g of longleaf seed.

Table 2. Fungicides rates applied to longleaf pine seed. Rates were based on 60 g of seed.

Fungicide	0.5x	1x	1.5x
Benlate®	0.075g	0.15g	0.225g
Dividend®	0.05mL	0.1mL	0.15mL
Manzate®	0.06g	0.12g	0.18g
Mertect®	0.0004mL	0.0008mL	0.0012mL
	1x	2x	3x
Zero-Tolerance®	1.0mL	2.0mL	3.0mL

Seed germination experiments

Germination chambers consisted of a plastic container which containing two pieces of KIMPAK® paper, moistened with 50 mL of sterile distilled water. Treatment with Benlate®, Dividend®, Manzate®, and Mertect® were replicated eight times, and Zero-Tolerance® treatments were replicated six times. Seed soaks of 10, 20, and 30 minutes were used for each of the three Zero-Tolerance® rates. After seed was treated, and allowed to dry, 40 seeds were placed on the moistened KIMPAK® in each chamber. Chambers were placed on a laboratory benches in a completely randomized design. Trials were maintained for 4 weeks, in a 12 hour photoperiod, and germination counts were taken weekly. Seeds no longer in contact with the KIMPAK® were considered germinated and removed from chambers.

RESULTS

The effect of the 11 chemicals on fungal growth can be separated into three distinct groups based on the inhibition of *Fusarium* growth on amended and non-amended media. Fungicides reacted by either completely inhibiting growth i.e., fungicidal, or allowing mycelial growth, fungistatic. Chemicals which were fungicidal on amended media and did not result in fungal growth when transferred onto non-amended media were characterized as biologically significant.

Treatment with Apron® (metalaxyl) did not affect *Fusarium* growth. Agar amended with Agribrom®, Baytan® (tiradimenol), Heritage® (azoxystrobin), and Tilt® (propiconazole) had a moderate effect on *Fusarium* growth. Overall, five fungicides (Benlate®, Dividend®, Manzate®, Mertect®, and Zero-Tolerance®) inhibited the fungal growth on amended media and inhibition persisted when fungi were placed on non-amended media.

The fungicides used in these experiments did not reduce the germination of longleaf pine seed. Seed treated with Benlate® had significantly greater germination than the untreated controls (Fig. 1a). Controls had 77% germination, the 0.5x rate had 86% germination, and 1.0x and 1.5x rates had 87%, respectively, for Benlate® treatments (Fig. 1a). In studies with Manzate®, nontreated controls had 78% germination, 85%, 88%, and 86% germination resulted from treatment with Manzate® for 0.5x, 1.0x, and 1.5x, respectively (Fig. 1c).

Dividend®, however, resulted in a trend towards decreasing germination as chemical rates increased (Fig. 1b). Untreated controls had 83% germination while the 0.5x, 1.0x, and 1.5x had 81%, 81%, and 79% germination, respectively. Seed treatment with Mertect® did not result in any change in germination (Fig. 1d) as controls had 80% germination while 0.5x-1.5x had 84%, 78%, and 79% germination rates, respectively. The use of the sterilant, Zero-Tolerance®, had no effect on longleaf pine germination by either rate of chemical applied or length of soak (Fig. 2).

Five of the 11 chemicals examined (Table 1) in these experiments, Benlate®, Dividend®, Manzate®, Mertect®, and Zero-Tolerance® significantly reduced the growth of each of the four *Fusarium* sp. tested. Subsequent germination trials conducted using these five chemicals indicate that Benlate®, Manzate®, and perhaps Zero-Tolerance® compounds could be utilized as seed treatments in longleaf containerized nurseries without detrimental effects.

In these trials treatment of longleaf seed with benomyl increased germination by 10% over untreated controls. Other studies by Weyerhaeuser using benomyl reported significantly lower recovery of *Fusarium* from benomyl treated longleaf seed from non-treated controls. The Weyerhaeuser study also reported that germination decreases as benomyl concentration increases from 0-10% active ingredient. While rates, and thus active ingredients were not identical, the current trials differ from the Weyerhaeuser findings since increasing benomyl rates did not affect longleaf pine germination as treatment with benomyl increased seed germination by 10% between untreated controls and treated seed (Fig. 1a). Pine seedling nurseries in North Carolina currently use benomyl as a pre-sowing seed treatment to control *Fusarium* on longleaf pine seed. However, North Carolina is the only state with a label for the use of benomyl as a seed treatment on longleaf pine. Since benomyl

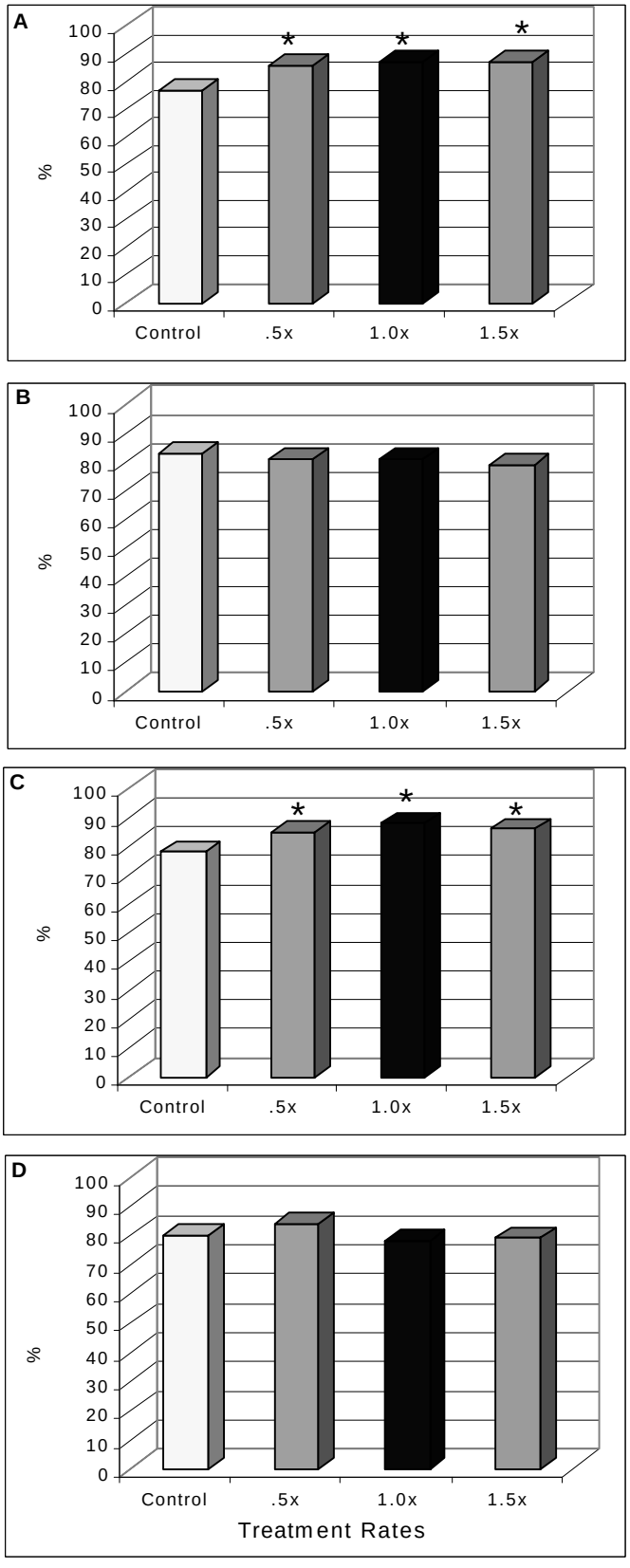

Figure 1. Germination of longleaf pine seed treated with **A)** Benlate, **B)** Dividend, **C)** Manzate and **D)** Mertect. Asterisk indicates significance over the control at the P=0.05 level.

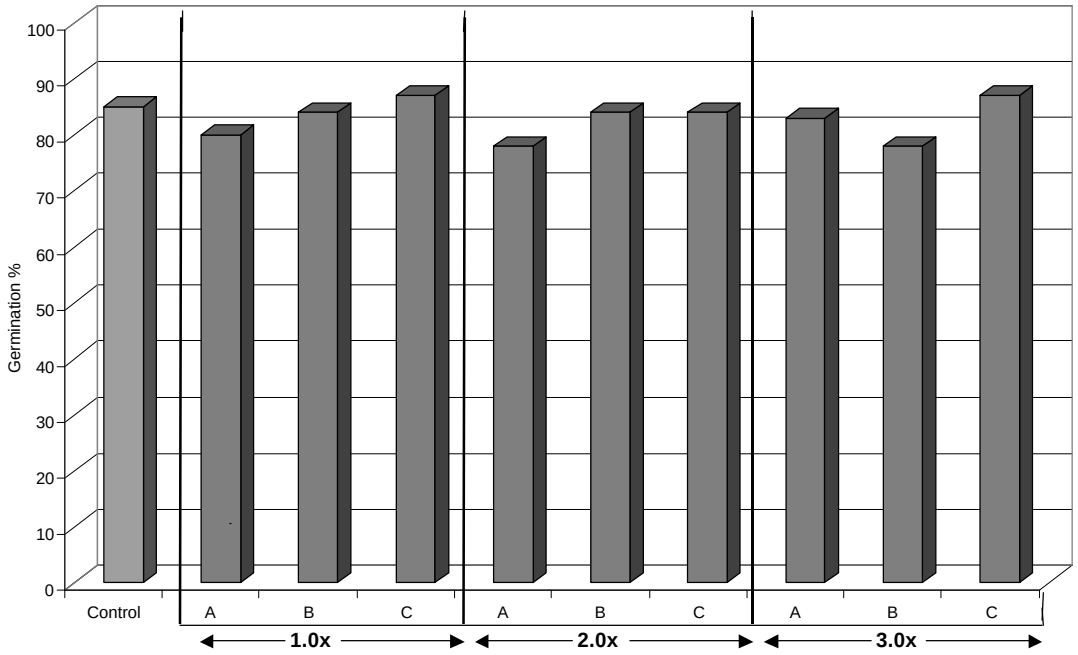

Figure 2. Longleaf pine germination treated with Zero-Tolerance. Letters above rate correspond to (A) 10 minute soak, (B) 20 minute soak, (C) 30 minute soak.

is formulated as a wettable powder the use of the fungicide in longleaf pine production could be easily added to pre-sowing procedures in a containerized operation.

Another potential compound for seed treatment was Manzate® . This chemical completely inhibited fungal growth on amended media, and this inhibition persisted when the original plug was transferred to non-amended media. Germination boxes that contained Manzate®-treated seed had 86% germination compared to 78% for the controls. Manzate® is labeled as a seed treatment for on a wide variety of agricultural crops for control of damping-off, seed decay, seedling blights and *Fusarium* on caprifig (*Ficus carica*) as well as seedborne *Fusarium* on potato (*Solanum tuberosum*) seed pieces.

MANAGEMENT IMPLICATIONS

Further studies need to be conducted with benomyl and Manzate on longleaf pine so that it may be possible to expand their labels to include treatment of longleaf seed for the production of longleaf seedlings throughout the southeastern United States. Currently, (January 2000), Dupont has forwarded a request from the Auburn University Southern Forest Nursery Cooperative to the Environmental Protection Agency to include South Carolina, Georgia and Alabama on the 24C label to use benomyl as a seed treatment on longleaf pine.