Auburn University
Southern Forest
Nursery Management
Cooperative

RESEARCH REPORT 02-8

COMPARING FUNGICIDE EFFECTS OF ARTIFICIAL MEDIA WITH CHANGES IN LONGLEAF SEED GERMINATION

by
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INTRODUCTION

Most nurseries that produce longleaf pine seedlings have had problems with at least some seedlots with poor germination and excessive seedling mortality. Fungicides or disinfectants can improve production but the loss of our most effective fungicide (benlate), and the expense and hazard associated with the alternative disinfectant (hydrogen peroxide) make it important to determine replacements. This study evaluates four alternatives. We evaluated five fungicides against four species of *Fusarium* commonly associated with longleaf pine seed and tested their effects on germination and seedling establishment in laboratory and greenhouse studies. Although fungicide evaluations on artificial media are relatively inexpensive and quick, their results often do not indicate how they will perform under natural conditions. We found that mean seed germination by fungicide treatment correlated with mean fungal growth (or lack of growth) for the same treatments after transfer from fungicide amended media but not for growth on amended media.

METHODOLOGY

Inhibition of fungi on artificial media: Four species of *Fusarium* (*F. circinatum* = *subglutinans*, *F. oxysporum*, *F. proliferatum* and *F. solani)* were transferred to malt yeast extract agar (MYE) amended with five rates of four fungicides or a disinfectant. Growth was measured after another 14 days on non-amended MYE. The four fungicides were, benomyl (Benlate 50WP, DuPont, Wilmington, DE, 50% a.i.), difenoconazole (Dividend 0.31 FS, Novartis, Greensboro, NC, 32.8% a.i.), mancozeb (Manzate 200DF, DuPont, Wilmington, DE, 75% a.i.), and thiabendazole (Mertect 340F, Merck, Rahway, NJ, 42.3% a.i.), and the disinfectant was hydrogen dioxide (Zero-Tolerance, BioSafe Systems, Glastonbury, CT, 23% a.i.). Except for benlate, which was labeled for longleaf

pine seed, rates for the other fungicides were estimated based on labeled rates for other crops. The hydrogen dioxide base rate was the recommended 1:50 dilution. Growth was measured after 14 days on the amended media and samples were re-transferred back to non-amended MYE to assess viability. The base rates for the fungicides is presented in Table 1. This entire study was carried out three times.

Fungicide effects on longleaf seed germination in the laboratory and greenhouse. The fungicide treatments used for seed were the three low rates used for artificial media. That is, either 0.5 , 1 or 1.5 times the base rate (Table 1). Treatments were prepared by mixing weighed amounts of benomyl, difenoconazole, or thiabendazole with 50 ml of sterile distilled water (SDW) or mixing mancozeb with 5 ml SDW. Seed were soaked for 10-minutes and then air-dried for 1 hr. Each fungicide treatment was repeated 8 times and the disinfectant treatments 6 times with 60 grams of seed treated per replicate. Seed from a 1996 bulk collection from Okaloosa County, Florida were treated and germinated in plastic chambers in the laboratory. Forty seed from each treatment replicate were placed on two pieces of germination paper moistened with 50 ml of SDW and kept at approximately 21°C for 4 weeks. Germinated seed were recorded and removed weekly and the study was repeated once. Seed from a 1998 bulk collection from the Bladen Lakes Seed Orchard, in NC was treated (as described above) and sown singly into of 85 cm^3 of ProMix in plastic container cells (Stuewe & Sons), covered with sand and kept in the greenhouse for 6 weeks. Seedlings were considered emerged when cotyledons were visible.

Table 1. Base rates (1 X rates) by fungicide used to amend malt yeast extract agar and to treat 60 g of longleaf pine seed.

Trade Name	Common Name	Product / 100ml agar	Product for 60 g Seed
Benlate 50 WP	benomyl	0.185 g	0.075 g
Dividend 0.31FS	difenoconazole	0.33 ml	0.05 ml
Manzate 200DF	mancozeb	0.20 g	0.06 g
Mertect 340F	thiabendazole	0.006 ml	0.0004 ml
Zero-Tolerance	hydrogen dioxide	1.0 ml	1.0 ml

Analysis: Fungal growth on fungicide-amended-media and on non-amended media after transfer from amended media were analyzed for the effects of fungicide (n=5), Fusarium species (n=4) and for species by fungicide interaction using SAS GLM. Where growth was greater than zero, on either media, the effects of fungicide rate were tested for linear trends using contrast statements. The effects of treatment (n=4) on germination were analyzed for the effects of treatment and seed source (which is confounded with germination medium) (n=2). Means for growth on amended and on non-amended media (see Table 2) were compared (for equivalent fungicide and rate) to mean germination for each seed source (see Table 3) using regression and correlation procedures.

RESULTS AND DISCUSSION

Three systemic fungicides (benomyl, Mertect and Dividend) a prophylactic fungicide (mancozeb), and a disinfectant (hydrogen dioxide) were evaluated. Although systemics often are more effective

in the field than prophylactics, in this study benomyl was the only systemic to increase germination of longleaf seed. Mancozeb was the only fungicide treatment after which no growth occurred among fungi transferred to media without fungicide. Along with benlate, only mancozeb significantly improved germination. Although treating longleaf seed with the disinfectant hydrogen peroxide has increased germination, hydrogen dioxide stopped fungal growth on media but did not improve germination. It appears that the high rates of hydrogen dioxide were toxic to seed and effected germination directly.

Among qualitative variables (species and fungicide) fungal growth differed by fungicide, by species, and for the interaction of fungicide and species both for growth on media with or without fungicide. No fungal growth occurred on benlate or mancozeb amended media and no growth occurred for transfers from mancozeb amended media (Table 2) precluding quantitative analyses for rate among these treatments. The benomyl and mancozeb treatment effects were linear with dose for the rates treated and this indicates that the treatments (dose rates) tested were in the proper range. Treatments that did not produce linear effects were not effective or perhaps not tested in adequate dosages.

Table 2. Mean average diameter (mm) for four species of fusarium by fungicide and rate after 14 days on MEA media with fungicide (amended yes) and after 14 days on that media without fungicide (amended no).

		Fungicide Rate					PR > F		
Treatment	Amended	1	2	3	4	5	Rate	Species	Linear
Benlate	Yes	0	0	0	0	0	NA	NA	NA
	No	60	32	18	7	15	0.01	0.01	0.01
Dividend	Yes	15	14	13	13	12	0.45	0.01	0.63
	No	81	81	79	78	76	0.01	0.01	0.01
Mancozeb	Yes	0	0	0	0	0	NA	NA	NA
	No	0	0	0	0	0	NA	NA	NA
Mertect	Yes	4	4	4	4	4	0.79	0.01	0.98
	No	81	81	80	79	79	0.39	0.01	0.18
hydrogen dioxide	Yes	1.4	0	0	0	0	0.18	0.19	0.08
	No	16	0	0	0	0	0.01	0.01	0.01

Three rates of five fungicide treatments used on seed (rates 1,2, and 3) plus a control produced 16 means for each seed source (Table 3). These germination means were analyzed for correlation with the 16 means for fungal growth on media with the equivalent treatment rates (1,2, and 3) and for growth after transfer to media without fungicide (Table 2). Although germination of neither seed source correlated ($p>0.05$) with growth on media containing fungicide, germination did correlate with growth after transfer from that media ($p < 0.01$). The regression for fungal growth after transfer from media with fungicide with germination by seed source is presented in Figure 1.

Table 3 Percent germination by seed source and fungicide rate with statistics for the GLM and linear models.

Treatment	Seed	Fungicide Rate 0	1	2	3	PR > F† Rate	Linear
Benlate	NC	11.3	10.0	12.1	15.6	0.06	0.03
	FL	32.2	34.5	34.7	35.0	0.12	0.03
Dividend	NC	11.3	9.0	9.7	8.6	0.59	0.28
	FL	32.2	32.4	32.2	31.5	0.48	0.18
Mancozeb	NC	11.3	12.3	18.4	17.2	0.03	0.01
	FL	32.2	33.9	35.2	34.3	0.006	0.002
Mertect	NC	11.3	9.7	8.2	10.0	0.82	0.61
	FL	32.2	33.7	33.0	31.8	0.86	0.52
hydrogen dioxide	NC	11.3	14.2	12.1	10.1	0.61	0.59
	FL	32.2	33.5	32.9	33.2	0.01	0.92†

† PR > F's in NC seed rows are for NC seed, those in the FL seed rows are for the combined NC + FL seed receiving that treatment.

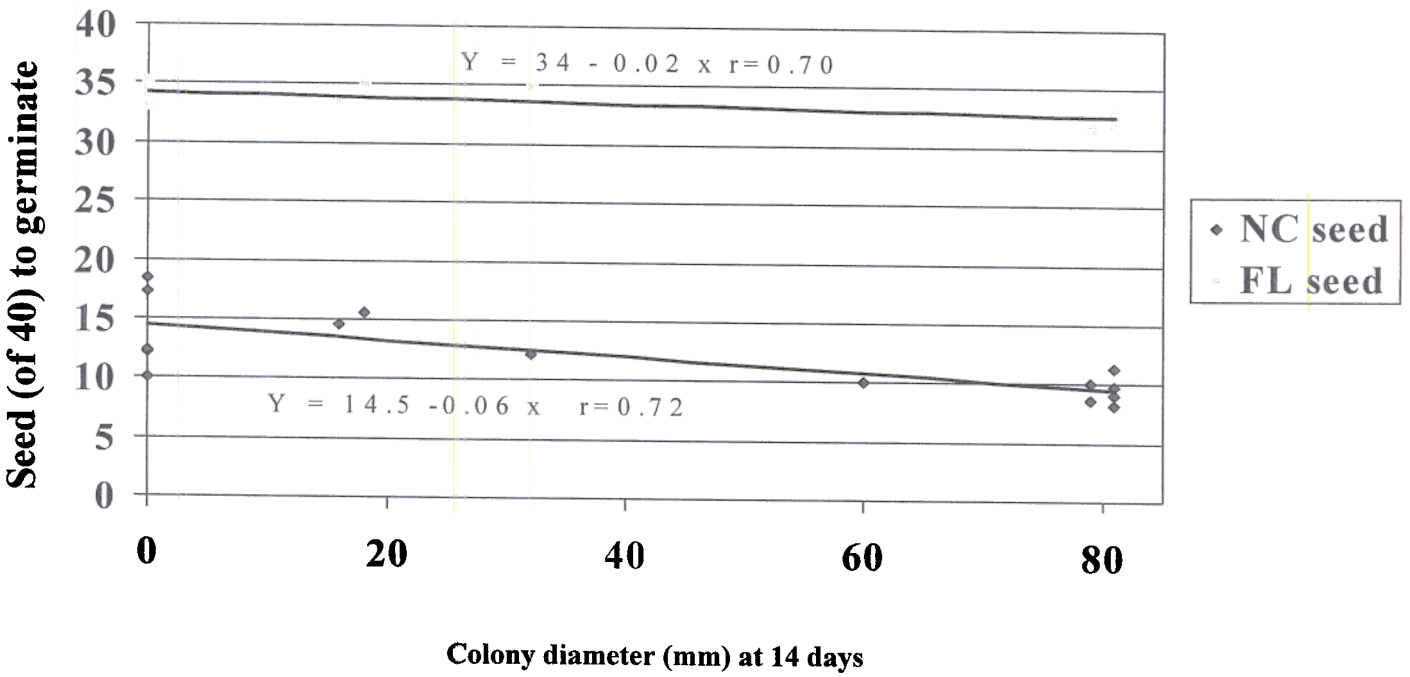

Figure 1. Longleaf seed germination by fungicide effects on fungal growth on media

MANAGEMENT IMPLICATIONS

Among the tested fungicides, mancozeb (along with benomyl) produced the greatest improvement in germination. The other fungicides and the disinfectant may have been at inappropriate rates. The relationship between the effectiveness of treatments on the ability of fusaria to resume growth after treatment on artificial media and the effectiveness of these treatments to improve germination needs further investigation. If this relationship holds up it will greatly enhance the speed and cost at which fungicides could be evaluated.