Effects of Thiabendazole-DMSO Treatment of Longleaf Pine Seed Contaminated with *Fusarium subglutinans* on Germination and Seedling Survival

G. B. RUNION, Former Graduate Research Assistant, and R. I. BRUCK, Associate Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

ABSTRACT

Runion, G. B., and Bruck, R. I. 1988. Effects of thiabendazole-DMSO treatment of longleaf pine seed contaminated with *Fusarium subglutinans* on germination and seedling survival. Plant Disease 72:872-874.

A thiabendazole-dimethyl sulfoxide soak increased in vitro germination of longleaf pine seed known to be contaminated with the pitch canker fungus, *Fusarium subglutinans*. Thiabendazole also resulted in lower percentages of seed and seedlings contaminated with the fungus. Thiabendazole was applied as Mertect 340F at concentrations of 423, 1,268, 2,114, 2,537, 3,382, or 4,228 μg a.i./ml and as Arbotect 20S at concentrations of 266, 798, 1,330, 1,596, 2,128, or 2,660 μg a.i./ml. Fungicides were suspended in 10% dimethyl sulfoxide to enhance efficacy. The 2,660 and 3,382 μg a.i./ml concentrations resulted in the highest germination rates for the Arbotect and Mertect treatments, respectively, but were not significantly different from most concentrations tested. All treatments reduced the recovery of *F. subglutinans* from seed that did not germinate and from seedlings compared with controls. The higher concentrations tended to be most effective. Arbotect was the more effective of the two formulations tested. The 2,660 μg a.i./ml treatment resulted in the highest germination rate and the lowest recovery of *F. subglutinans* from seedlings.

Pitch canker, caused by *Fusarium subglutinans* (Wollenw. & Reink.) Nelson, Toussoun, & Marasas (= *F. moniliforme* Sheld. var. *subglutinans* Wollenw. & Reink.) (18), was first reported on longleaf pine (*Pinus palustris* Mill.) in 1947 (14). The disease was not considered economically important at that time and the fungus even was tested for its ability to increase resin flow in longleaf pine to benefit the naval stores industry (22).

Pitch canker is now known to cause damage on most southern pine species (11). *F. subglutinans* causes cankers and/or branch dieback in seed orchards of most southern pine species, including longleaf pine (3,9–11). The fungus also is known to infect and damage the seed, cones, and other reproductive structures of these southern pines (1,3,15,16), and to cause damping-off of longleaf (19) and other (4) pine seedlings.

Present address of first author: School of Forestry, Auburn University, AL 36849-4201.

Use of trade names does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned.

This research was supported in part by a grant from Merck & Co., Inc., Rahway, NJ.

Paper No. 11280 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695-7601.

Accepted for publication 12 May 1988 (submitted for electronic processing).

In 1984, poor germination of longleaf pine seed and high incidence of seedling damping-off were observed for seed produced by the North Carolina Division of Forest Resources' (NCDFR) seed orchard at Bladen Lakes State Forest. In a preliminary test, *F. subglutinans* was isolated on selective medium (17) from nondisinfested seed (197 of 200) and from seed that were surface-disinfested by dipping in 95% ethanol and flaming (169 of 200). This preliminary test indicated that the fungus was contained both externally on and internally in these seed, which will be referred to as contaminated to reflect both conditions. The fungus also was isolated from seedlings (40 of 40) that displayed damping-off symptoms.

Benzimidazole fungicides are known to be effective against *Fusarium* spp., including *F. subglutinans* (5). Benomyl was effective in eradicating *F. moniliforme* Sheld. and *F. oxysporum* Schlecht. from asparagus (*Asparagus officinalis* L.) seed (7). Benomyl (12) and thiabendazole (20) can suppress growth of *F. subglutinans* in culture, and thiabendazole is effective in reducing the impact of pitch canker on loblolly pine (*P. taeda* L.) seedlings (20).

The objective of this research was to determine the effects of two formulations of thiabendazole, suspended in 10% dimethyl sulfoxide (DMSO), on germination of longleaf pine seed contaminated with *F. subglutinans* and on the subsequent survival of these seedlings.

MATERIALS AND METHODS

Longleaf pine seed were obtained from the NCDFR seed orchard located on Bladen Lakes State Forest, near Elizabethtown, NC. A bulked seed collection was used to avoid possible clonal bias in sensitivity to the fungicides, germination, or susceptibility to the fungus. Clonal variation in disease incidence (0–75% of ramets exhibited infected terminals) among the 32 orchard clones had been observed (G. B. Runion, *unpublished*).

Seed were washed under rapidly running tap water for 6 hr before treatment in an effort to reduce surface contaminants without affecting germination. Thiabendazole was applied as Mertect 340F (42.28% a.i.) at concentrations of 423, 1,268, 2,114, 2,537, 3,382, or 4,228 μg a.i./ml and as Arbotect 20S (26.6% a.i.) at concentrations of 266, 798, 1,330, 1,596, 2,128, or 2,660 μg a.i./ml. Preliminary experiments had defined the maximum concentrations of each formulation, above which phytotoxic effects on germination were observed.

Treatments were applied by soaking 90 seed in 100 ml of suspension containing each fungicide concentration, 10% DMSO by volume, and sterile distilled water. Preliminary experiments demonstrated that DMSO was required for the fungicides to be effective. The DMSO concentration was established during this preliminary experiment. Control seed were soaked in 100 ml of solution containing 10% DMSO in sterile distilled water. Seed were soaked for 12 hr at 40 C on a rotating shaker (approximately 90 rpm) to keep the fungicides in suspension. Preliminary experiments were used to define the optimum duration of seed soak.

After the 12-hr soak, seeds were rinsed for 10 min in sterile distilled water and placed individually in sterile moist chambers made from test tubes (16× 150 mm) containing filter paper strips to support and provide moisture to the seeds. Test tubes contained 5 ml of sterile distilled water and were sealed with permeable membrane caps.

Thirty test tubes were placed in wire mesh baskets and three baskets per treatment were incubated in a completely ramdomized design under continuous flourescent light at 25 C. Seed were maintained under these conditions for approximately 6 wk, at which time the number of germinated seed (seedlings) was recorded. All seed and seedlings were removed from the test tubes, surface-disinfested in 0.525% NaOCl for 5 min,

and placed on Nash and Snyder's *Fusarium* selective medium (17). The seed and seedlings were incubated under ambient laboratory conditions (approximately 20 C) for 10 days, at which time the presence or absence of *F. subglutinans* was determined for each seed and seedling by examination of fungal characteristics (18) using a compound microscope. The experiment was conducted three times using seed from the same collection in each run.

Data taken included the number of seed that germinated and the numbers of seedlings and seed that yielded *F. subglutinans* in culture. All numbers were expressed on a percentage basis for analysis. Mean to variance plots for all data demonstrated no heterogeneity of variance and, thus, data were not transformed before analysis. Data were analyzed using the General Linear Models procedure (PROC GLM) and the Regression procedure (PROC REG) of the Statistical Analysis Systems (21). In all analyses, means were considered significantly different if they differed at the $P < 0.05$ level.

RESULTS

There were no significant differences among the three runs of the experiment for any variable. Therefore, data presented are the average of all runs. All concentrations of Arbotect, except 266 μg a.i./ml, and all concentrations of Mertect, except 423 and 2,114 μg a.i./ml, resulted in higher seed germination rates than did controls. The 2,660 and 3,382 μg a.i./ml concentrations exhibited the greatest percent germination for the Arbotect and Mertect treatments, respectively. However, percent germination for these best treatments was not significantly different from germination for most concentrations tested (Table 1). Percent germination data for both formulations were not adequately described by any model under regression analysis (R^2 values < 0.25).

All concentrations of both thiabendazole formulations resulted in less frequent recovery of *F. subglutinans* from germinated longleaf pine seed (i.e., seedlings) and from seed that did not germinate compared with controls (Table 1). The 2,660 and 4,228 μg a.i./ml concentrations exhibited the lowest recovery of *F. subglutinans* from seedlings for the Arbotect and Mertect treatments, respectively. The 1,596 and 4,228 μg a.i./ml concentrations exhibited the lowest recovery of the fungus from seed that did not germinate for the Arbotect and Mertect treatments, respectively. However, recovery of *F. subglutinans* from seedlings and from seed for these best treatments was not significantly different from recovery observed for most concentrations tested (Table 1). The percentage of seedlings infected and the percentage of seed contaminated with *F. subglutinans* demonstrated significant (R^2 values of 0.73–0.79) linear responses to the natural logarithm of the thiabendazole concentration for both formulations tested.

DISCUSSION

This study indicates that most concentrations of both thiabendazole formulations, when used with DMSO, increased germination of and reduced recovery of *F. subglutinans* from longleaf pine seed and seedlings in vitro. Further experimentation is required to determine whether similar effects of the fungicides would occur under greenhouse or nursery conditions. The potential for a seed soak treatment to be effective in the field seems possible as many of the control seedlings exhibited signs and symptoms of damping-off while these signs and symptoms existed on very few thiabendazole-treated seedlings.

Preliminary studies indicated that DMSO was necessary in order to obtain efficacy of the fungicides. This is supported by work on asparagus (7) and *Brassica* spp. (13) where benomyl in acetone, as a solvent, was more effective in eradicating *Fusarium* spp. than benomyl in water. Acetone, a lower concentration of DMSO, or other solvents might provide similar or more effective results than observed in this study, and deserve further investigation We did not determine the physiological basis of the increased efficacy by DMSO or the site and mode of action of thiabendazole in longleaf pine seed, but these subjects should be explored.

In general, Arbotect was more effective than Mertect at similar, or even lower, concentrations of thiabendazole for all variables measured. Differences in solubility between Arbotect (which is acidified to enhance its solubility in water) and Mertect (a less soluble colloidal suspension) (2) is the most probable reason for the differences in efficacy observed between the two fungicides. We attempted to minimize the difference in solubility by soaking seed on a rotating shaker and by using DMSO, because the solubility of thiabendazole increases in DMSO (2).

Percent germination was increased by most concentrations of both thiabendazole formulations tested. However, there were very poor correlations between thiabendazole concentration and seed germination. These poor correlations may have resulted from nonuniform bulking of seed from the 32 orchard clones that could have provided differences in sensitivity to the fungicides, germination, or numbers of contaminated, empty, or nonviable seed. Use of seed from one or a few clones and elimination of empty and excessively damaged seed, through use of radiography (16) or other techniques (gravity table or air separator), may increase the correlation of percent germination to thiabendazole concentration.

F. subglutinans is known to survive as a soil inhabitor (6) and has been isolated from pine plantation and seed orchard soil (8). Seed that do not germinate and are infested with the fungus could serve as sources of inocula and increase the incidence of damping-off in nursery beds. Therefore, reduction in the number of seed infested with *F. subglutinans* could

Table 1. Effects of thiabendazole seed soak treatments on germination of longleaf pine seed in vitro[v]

Thiabendazole formulation	Thiabendazole concentration (μg a.i./ml)	Percent germination[w]	Percent seedlings infected[x]	Percent seeds contaminated[y]
Control	0	38.2 c[z]	77.9 a	78.2 a
Arbotect	266	35.6 c	17.2 bc	19.1 bc
	798	52.2 ab	9.6 cd	12.1 cd
	1,330	57.0 a	17.5 b	22.7 b
	1,596	45.7 b	11.1 bcd	9.8 d
	2,128	53.0 a	9.9 cd	10.5 cd
	2,660	57.4 a	7.9 d	16.0 bcd
Mertect	423	44.1 bc	25.3 b	19.0 bc
	1,268	48.2 ab	18.7 bc	21.5 b
	2,114	44.4 bc	17.9 bc	17.4 bc
	2,537	54.4 a	13.6 c	15.4 bc
	3,382	55.6 a	14.1 c	12.9 c
	4,228	48.5 ab	12.1 c	12.0 c

[v] Treatments were applied by soaking seed for 12 hr in the various thiabendazole concentrations plus 10% DMSO. Control seed were soaked in distilled water plus 10% DMSO.

[w] Data are based on 90 seed per run of the experiment, and represent the average of three runs.

[x] Data are based on the percentage of seed that germinated and yielded *Fusarium subglutinans* in culture, and represent the average of 96–155 seedlings for all three runs of the experiment combined.

[y] Data are based on the percentage of seed that did not germinate but yielded *F. subglutinans* in culture, and represent the average of 115–174 seed for all three runs of the experiment combined.

[z] Within a formulation, numbers within a column followed by the same letter are not significantly different according to contrasts using the General Linear Models procedure of the Statistical Analysis System. All concentrations of both formulations are compared with the same control.

be an important additional benefit of a thiabendazole seed soak treatment.

Finally, this type of seed treatment is a preliminary step in addressing infection after it has occurred, and is an attempt to ameliorate the situation temporarily so that a crop of longleaf pine can be raised from contaminated seed. Although little is known at present concerning the epidemiology of this aspect of pitch canker in general, and the mechanisms of seed infection in particular, we must address the causes of longleaf pine seed infection and not merely treat the result.

ACKNOWLEDGMENTS

Appreciation is extended to K. O. Summerville for providing the longleaf pine seed used in the study and to Glen Stanosz for advice and assistance in implementing the in vitro germination technique.

LITERATURE CITED

1. Anderson, R. L., Belcher, E., and Miller, T. 1983. Occurrence of internal seed fungi in slash pine seed produced in seed orchards in the United States. 20th Congress, Int. Seed Test. Assoc., Ottawa, Canada, Reprint 15. 10 pp.
2. Anonymous. 1983. Tecto/Mertect Technical Bulletin. 3rd ed. MSD Agvet Div., Merck & Co., Inc. Rahway, NJ.
3. Barrows-Broaddus, J., and Dwinell, L. D. 1985. Branch dieback and cone and seed infection caused by *Fusarium moniliforme* var. *subglutinans* in a loblolly pine seed orchard in South Carolina. Phytopathology 75:1104-1108.
4. Bloomberg, W. J. 1981. Disease caused by *Fusarium* in forest nurseries. Pages 178-187 in: *Fusarium*: Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania University Press, University Park, PA. 457 pp.
5. Bolkan, H. A., Dianese, J. C., and Cupertino, F. P. 1978. Chemical control of pineapple fruit rot caused by *Fusarium moniliforme* var. *subglutinans*. Plant Dis. Rep. 62:822-824.
6. Bolkan, H. A., Dianese, J. C., and Cupertino, F. P. 1979. Survival and colonization potential of *Fusarium moniliforme* var. *subglutinans* in soil. Phytopathology 69:1298-1301.
7. Damicone, J. P., Cooley, D. R., and Manning, W. J. 1981. Benomyl in acetone eradicates *Fusarium moniliforme* and *F. oxysporum* from asparagus seed. Plant Dis. 65:892-893.
8. Dwinell, L. D., and Barrows, J. B. 1978. Recovery of the pine pitch canker fungus from pine plantation and seed orchard soil. (Abstr.) Phytopathol. News 12:207.
9. Dwinell, L. D., and Barrows-Broaddus, J. 1981. Pitch canker in seed orchards. Pages 234-240 in: Proc. South. For. Tree Improv. Conf. 16th. Blacksburg, VA. 363 pp.
10. Dwinell, L. D., and Barrows-Broaddus, J. 1983. Pine wilt and pitch canker of Virginia pine in seed orchards. Pages 55-62 in: Proc. South. For. Tree Improv. Conf. 17th.
11. Dwinell, L. D., Barrows-Broaddus, J. B., and Kuhlman, E. G. 1985. Pitch canker: A disease complex of southern pines. Plant Dis. 69:270-276.
12. Dwinell, L. D., Kuhlman, E. G., and Blakeslee, G. M. 1981. Pitch canker of southern pines. Pages 188-194 in: *Fusarium*: Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania University Press, University Park, PA. 457 pp.
13. Harman, G. E., and Nash, G. 1978. Soaking *brassica* seeds in fungicide solutions to eradicate seedborne fungi: A comparison of aqueous and organic solvent techniques. Plant Dis. Rep. 62:408-412.
14. Hepting, G. A. 1947. Stimulation of oleoresin flow in pines by a fungus. Science 105:209.
15. Mason, G. N., and Van Arsdel, E. P. 1978. Fungi associated with *Pinus taeda* seed development. Plant Dis. Rep. 62:864-867.
16. Miller, T., and Bramlett, D. L. 1979. Damage to reproductive structures of slash pine by two seed-borne pathogens: *Diplodia gossypina* and *Fusarium moniliforme* var. *subglutinans*. Pages 347-355 in: Proceedings Flowering and Seed Development in Trees: A Symposium. F. Bonner, ed. U.S. Dep. Agric. For. Serv., South. For. Exp. Stn., New Orleans, LA. 515 pp.
17. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
18. Nelson, P. E., Toussoun, T. A., and Marasas, W. O. F. 1983. *Fusarium* Species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park, PA. 193 pp.
19. Pawuk, W. H. 1978. Damping-off of container grown longleaf pine seedlings by seed borne *Fusaria*. Plant Dis. Rep. 62:82-84.
20. Runion, G. B., and Bruck, R. I. 1988. The effects of thiabendazole on *Fusarium subglutinans*, the causal agent of pitch canker of loblolly pine. Plant Dis. 72:297-300.
21. SAS Institute Inc. 1982. SAS User's Guide: Statistics. Statistical Analysis Systems (SAS) Institute Inc., Cary, NC. 584 pp.
22. True, R. P., and Snow, A. G., Jr. 1949. Gum flow from turpentine pines inoculated with the pitch canker *Fusarium*. J. For. 47:894-899.