ROOT ROT AND DAMPING-OFF

CONTAINER-GROWN SOUTHERN PINE SEEDLINGS 1/

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Abstract.--Fusarium spp. were found to be a major cause of root rot and damping-off of greenhouse grown containerized southern pine. Pythium and Rhizoctonia were associated with mortality in transit. Healthy appearing seedlings associated with diseased seedlings exhibited poor field survival. The major source contamination is from Fusarium naturally present on the seed coats of longleaf seed. In inoculation studies with Fusarium, Pythium and Rhizoctonia a Truban-Benlate combination gave better control than did Captan or Busan-72.

INTRODUCTION

Wilting and dying of pine seedlings were reported at the Stuart Containerized Seedling Project in Louisiana during April of 1972. The seedlings were part of a pilot project by Region 8 of the U. S. Forest Service to adapt containerization to southern pine species. Loblolly, slash, shortleaf and longleaf pines were being grown in greenhouses in paper pots with a commercial potting medium of peat, perlite, and vermiculite. Wilting and dying were most prevalent on longleaf seedlings, but the other species were also affected.

Symptoms on longleaf included various degrees of root rot, absence of lateral roots, and rotting of the seedling at the root collar. Because of the grass stage characteristic of longleaf seedlings, cotyledons and needles were often rotted near the soil surface. Symptoms on the other species included root rot and stem lesions at the root collar. Fusarium spp. consistently grew from diseased tissue placed on cultural media and were found sporulating on diseased seedlings.

In an effort to control the disease, the seedlings were drenched with a Captan-Terrachlor mixture. Two weeks later the seedlings were shipped to various National Forests in the South for outplanting.

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About the first of May another seedling crop was started. Captan drenches were applied weekly starting about the fifth week after seeding. In spite of this the disease began to develop, in some boxes of seedlings, primarily in those seeded with longleaf pine. Again, Fusarium was isolated from the diseased seedlings.

At the end of the growing period, about eight weeks after seeding, those seedlings appearing to be healthy were shipped in refrigerated trucks at 55° F. to the forests for outplanting. Longleaf seedlings in one shipment to Florida began to die shortly after they arrived. Extension personnel at the University of Florida implicated Pythium and Rhizoctonia as causing the mortality, and suggested better ventilation of the seedlings in transit.

Following the development of the disease studies were initiated to:

1. Determine the effectiveness of the fungicide drench
2. Locate sources of contamination Test fungicides for controlling the disease.

METHODS

Effect of drenching.--Following drenching, the seedlings seemed somewhat improved in that the rate of mortality had apparently slowed down. To evaluate the effectiveness of the drenching, 200 seedlings of each species were outplanted on April 20, 1972, in four blocks of 50 seedlings per block. The seedlings were observed periodically until October 3, and mortality was recorded at each observation.

Sources of contamination.--In an effort to determine the sources of contamination, isolations were made onto culture media from the potting soil, water used in watering the seedlings, and soil outside the greenhouse which might be blown or otherwise transported into the greenhouse.

During the summer, no seedlings were grown because of the excessive heat in the greenhouses. In October a new crop was seeded. To get a better understanding of disease development, the seedlings were observed weekly. It soon became evident that early disease development was associated with seed coat retention. The disease first appeared on the cotyledons of seedlings which had failed to cast their seed coats; it then spread down the cotyledons to the stem eventually killing the affected seedlings. During moist overcast weather, fungus growth could be seen on the cotyledons. Examination of the fungi indicated Fusarium spp. were present on all diseased seedlings.

These observations indicated that one major source of contamination was from fungi naturally present on the seeds being used. Therefore, 25 seeds from each of five longleaf seed sources (Texas, Louisiana, Mississippi, Alabama, and South Carolina) were washed with sterile water, and the water placed on a medium selective for Fusarium. In addition, 25 seeds from each seed source were placed directly on the same medium to determine whether Fusarium spp. are naturally present on longleaf seeds.

Fungicide testing.--Growing large numbers of seedlings in a small space and under greenhouse facilities creates ideal conditions for disease development. The root rot and damping-off experienced attests to this fact. Although drenches of Captan and later Busan were applied once the disease became evident, they were not highly effective in controlling the disease. For this reason, fungicide evaluation tests were conducted in an attempt to find a treatment that would control Fusarium, Pythium, and Rhizoctonia in container grown southern pine seedlings. Since longleaf pine was most susceptible to the disease, it was used in the evaluation.

Three fungicides were evaluated in the study: (1) Captan (a 50 percent wettable powder of the active ingredient N-[(trichloro-methyl)thio]-4-cyclohexene-1,2-dicarboximide); (2) Busan-72 (a 60 percent emulsifiable liquid of 2-(thiocyanomethylthio)benzothiazole); and (3) a mixture of Truban and Benlate. Truban is 30 percent wettable powder consisting of the active ingredient 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole. Benlate, also registered as benomyl, is a 50 percent wettable powder of methyl-1(butylcarbamoyl)2-benzimidazolicarbamate.

Captan has been the standard for the control of seedling diseases for a number of years. More recently, Busan-72 has been registered for protecting pine seedlings grown in plastic bullets. Busan is reported to be effective against Rhizoctonia, Pythium, and Fusarium fungi. Truban and Benlate are new fungicides that have been reported to work well in combination. Truban is effective against diseases caused by Pythium and hytophthom and Benlate is more effective on Rhizoctonia and Fusarium.

These three fungicide treatments were applied at the recommended levels to control diseases of containerized seedlings caused by Fusarium, Pythium, and Rhizoctonia. All fungicides and fungi were mixed with the medium before filling the containers. Busan-72 was mixed with a quantity of water that could be dispersed evenly throughout the media. The rates of application for each fungicide were: (1) Busan-72, 1 ml per 5.2 gallons applied at about 1 ml per container; (2) Captan, 554 mg of the formulation mixed per cubic foot of medium; and (3) Truban-Benlate, 2000 and 660 mg, respectively, mixed per cubic foot of medium.

The fungi were grown for two weeks on rye grain prior to mixing into the potting medium. Twenty-five ml of infested grain were incorporated into each fungus treatment. Where fungi were mixed in combination, each was applied at the same 25 ml rate. In all, each fungicide was tested against each individual fungus and all possible mixed combinations of the fungi.

Three replications or blocks were tested for each treatment combination. Each treatment replication consisted of 266 Japanese paper pots (3x15 cm) in a weighed cardboard box filled with a commercial medium consisting of peat, perlite, vermiculite, and nutrients. Seeds used in the study were soaked in a 3-percent solution of hydrogen peroxide for 24 hours prior to use to remove fungi present on the seedcoats. The seedlings were grown for 10 weeks before the final evaluation was made.

Two types of measurements were made: (1) toxicity effects as determined by seed germination on the medium; (2) evaluation of disease control by loss of germinated seedlings and reduction in seedling development. An additional determination of field survival and growth was planned, but greenhouse mortality was so great that insufficient numbers of seedlings remained.

The effects of fungicide incorporation into the growing medium was determined by the toxicity to germinating seeds. Percent germination was measured on the medium in the containers under greenhouse conditions and by tests conducted under controlled conditions in the seed testing laboratory. Duplicate 100-seed samples were tested in the laboratory on the same medium as used in the greenhouse tests.

Upon completion of the growing period, a determination of the number of established seedlings remaining in the tubes was made. Although it was planned to sample 100 seedlings per box for total dry weights, heavy mortality forced a reduction in the number per sample to 10. In some treatments no seedlings survived and therefore no statistical analyses of dry weight data could be made.

RESULTS

Effect of drenching--Mortality occurred in all species that were outplanted. Most of this occurred during the first two to four weeks (fig. 1). After two weeks, over 30 percent of the longleaf and loblolly seedlings were dead. Mortality in shortleaf and slash seedlings during this period was slower, reaching about 30 percent for shortleaf and 15 percent for slash. By October, only slash pine showed significantly better survival (82 percent) than the other species. No differences in survival were present when loblolly (62 percent), shortleaf (61 percent) and longleaf (58 percent) were compared.

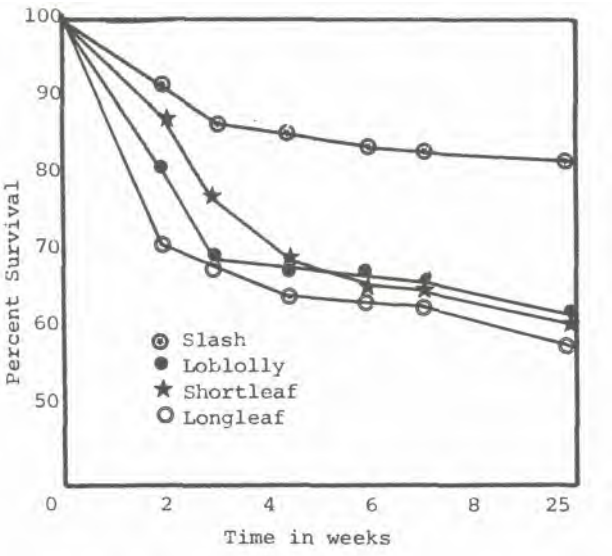

Figure 1.--Field survival of container grown southern pine seedlings grown in a greenhouse where Fusarium spp. caused root rot and damping-off.

Although no isolations were made from dead seedlings, several seedlings were examined in the field. Most of these had symptoms of root-rot or damping-off, and the mortality was believed to be due to disease since sufficient rainfall was present during this period.

Sources of contamination.--No organisms known to cause root rot or damping-off of southern pine were recovered from the potting mix or the water. Fusarium spp. were found in the soil outside the greenhouse and could be one source of contamination.

Examination of the longleaf seed on culture media showed that 8 - 20 percent of the seed from a given source was infested with Fusarium. In all, five species of Fusarium were recovered. Listed in the frequency with which they occurred, they were: F. moniliforme, F. oxysporum, F. solani, F. roseum, and F. tricinctum.

Representative cultures of these five Fusarium species were grown on rye grain and the infested grain mixed with the potting medium. Longleaf seeds which had been surface sterilized with a 24-hour soak in 3 percent hydrogen peroxide were germinated in the infested media. Three weeks after sowing, damping-off was observed in treatments containing F. oxysporum, F. solani, and F. moniliformi. No damping-off occurred in F. roseum or F. tricinctum treatment.

Fungicide testing.--Incorporation of the fungicides into the growing media had no adverse effect on initial germination of longleaf pine seeds. Greenhouse germination in uninoculated tubes for the Captan, Busan-72, Truban-Benlate, and no fungicide treatments averaged 74, 79, 76 and 79 percent, respectively, (table 1). Laboratory germination averaged 9 percentage points less (65, 65, 73, and 70 percent) for the same treatments. The reduced germination in the laboratory probably reflects some toxicity due to testing in closed germination dishes.

Although the fungicides had no marked effect on seed germination, the incorporation of Fusarium, Pythium, and Rhizoctonia inocula, singly and in combination, into the media did. Rhizoctonia resulted in the greatest reduction in germination when the three fungi were tested singly. Only 16 percent of the seeds germinated with no fungicide treatment, compared to 50 and 65 percent when inoculated with Pythium and Fusarium (table 1). The Fusarium-Pythium combination resulted in 62 percent germination, but all other combinations that contained Rhizoctonia resulted in germination below 15 percent.

The Truban-benlate fungicide treatment was the most effective in controlling losses to diseases during the germination period. This fungicide combination was particularly effective compared to Captan and Busan-72, in protecting germinating seedlings from Rhizoctonia (table 1). Fusarium did not result in important losses during the germination period even when unprotected by fungicides. There seemed to be no consistent differences in the effectiveness of Captan and Busan-72 in controlling disease organisms. However, they were both inferior to the Truban-Benlate treatment in controlling diseases, except for Captan with Pythium.

The same trends established during germination were found when seedling establishment was evaluated at 10 weeks. Truban-Benlate treatment was greatly superior to Captan and Busan-72 in

Table 1.--Treated growth and resulting seedling establishment and dry weights after 10 weeks growth in Japanese Paperpots filled with a media having incorporated fungicides and fungus inocula

Fungi treatments	Captan 50W			Busan-72			Truban-Benlate			No fungicides		
	Germ.	Estab. seedlings	Dry wt.	Germ.	Estab. seedlings	Dry wt.	Germ	Estab. seedlings	Dry wt.	Germ.	Estab. seedlings	Dry wt.
	Percent		Mg.	Percent		Mg.	Percent		Mg.	Percent		Mg.
Fusarium	70	34	105	67	34	104	69	39	95	65	44	150
Pythium	66	33	149	45	11	106	68	58	114	50	7	148
Rhizoctonia	8	2	--1/	6	0	--	53	19	94	16	2	--
Fus.-Pythium	46	6	136	60	3	147	75	43	100	62	7	142
Fus.-Rhizoctonia	12	0	--	8	0	--	56	18	93	9	1	--
Pythium-Rhiz.	8	0	--	15	0	--	52	25	116	12	0	--
Fus.-Pyth.-Rhiz.	23	0	--	7	0	--	48	8	82	10	0	--
Control	74	61	140	79	69	144	76	55	125	79	64	141

1/ Dashes indicate no data available.

protecting the seedlings. Captan was better than Busan-72 in controlling Pythium. Although the Truban-Benlate treatment was the most effective, seedling survival in Truban-Benlate control was only 55 percent compared to 61, 69, and 64 percent for the Captan, Busan-72, and no fungicide controls (table 1).

The limited data on seedling dry weights indicates that Truban-Benlate added to media could retard seedling development. Seedling dry weights averaged 125 mg when grown in media with Truban-Benlate incorporated, compared to an average of 142 mg for the other fungi controls.

DISCUSSION

Damping-off and root rot of container grown southern pine seedlings results in seedling mortality, causes a reduction in seedling growth and affects seedling survival following outplanting. Fusarium, Pythium, and Rhizoctonia have been associated with diseased seedlings. Of these fungi, Fusarium is most commonly found on diseased seedlings in the greenhouse and is believed to be the major cause of the disease. Species of fungi enter the greenhouse in various ways to contaminate seedlings. In the case of Fusarium, three species capable of causing damping-off were recovered from naturally infested longleaf seed demonstrating this to be an important source of contamination. Other contamination of the seedlings probably occurs from normal handling of the seedlings in the greenhouse. This would account for other genera such as Pythium or Rhizoctonia becoming established. However, in the greenhouse, Pythium has never been found to be a cause of mortality, and only one case of Rhizoctonia causing seedling mortality has been observed. In that case Rhizoctonia was present in four adjacent boxes and was controlled by drenching with fungicides. However, both Pythium and Rhizoctonia have been implicated with seedling mortality following shipment.

The fungicides tested had no adverse effect on germination of longleaf pine seeds unless tents were conducted in closed germination testing dishes. The incorporation of Fusarium, Pythium, and Rhizoctonia inocula into the media greatly reduced germination, even when the fungicides were also included.

However, the Truban-Benlate treatment was by far the most effective because the combination gave a wide spectrum coverage of disease organisms. Though Truban-Benlate did a superior job in controlling diseases, there were indications that it retarded seedling development.

Question: Do fungi affect bare-root stock of different species in the same degree as they do container stock?

Pawuk: I don't think I can answer that. The same fungi that are important in greenhouses also cause damage in nurseries.