Seed-protectant fungicides for control of Douglas-fir and ponderosa pine seedling root rots

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The USDA Forest Service nurseries at Wind River, Carson. Wash., and at Bend. Oreg.. have periodically suffered large losses of seedlings to root rot fungi. Several promising seed-protectant materials were evaluated in controlled environmental chamber tests for their effectiveness in preventing losses of Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) and ponderosa pine *(Pinus ponderosa* Laws) seedlings to pre- and post-emergence damping-off caused *by Pythium* and *Fusarium* spp. Of the seven materials tested only thiram and captan are currently registered for use as seed protectants on forest tree seeds (4).

Materials and Methods

Soils

Infested soil was obtained from nursery beds at the Wind River Nursery, Carson, Wash. and the Bend Nursery, Bend, Oreg. 1 to 2 weeks before use, and stored at 2° C. To insure uniformity of the fungal propagules throughout the samples, soils were individually screened and mixed prior to use. The soils were assayed for *Pythium and Fusarium* spp. according to the procedures outlined by Russell and Smith (personal communications).

The soil was placed in cylindrical ice-cream containers (8.5 cm by 15.2 cm) provided with drainage holes. A mixture of peat moss and vermiculite had been placed in the bottom of each container before adding the soil.

Seeds

Dewinged, nonstratified seeds were obtained from each nursery. Douglasfir seeds (Wind River Nursery) and ponderosa pine seeds (Bend Nursery) were X-rayed with a Faxitron, model 8050-010, on Kodak type M (8x10inch) safety film. The Douglas-fir and ponderosa pine seeds were exposed to 15 kV for 0.7 and 1.5 minutes. respectively. The seeds were exposed only once to minimize damage to the embryos (1). Seeds that appeared damaged or partially filled on the X-rays were discarded. Selected seeds were stratified by a cold soak in running water for 48 hours, towel dried, and stored in open polyethylene bags for 25 days at 2° C.

A test of seed viability was made after stratification. Fifty seeds of each species were placed on 250 ml of sponge Rok (Paramount Perlite Co.), premoistened with 40-42 ml of sterile distilled water, in 12.7 cm square transparent plastic containers. The seeds were exposed to light emitted from fluorescent lamps for 9 hours each day for 12 days. Tests were replicated four times for each species. Germination was considered complete when the hypocotyl was as long as or longer than the seed. Germination was 92.0 and 98.5 percent for Douglas-fir and ponderosa pine seeds. respectively.

Fungicidal Seed Treatments'

The formulations received from cooperating chemical companies were as follows:

42 percent thiram (tetramethylthiuram disulfide) (Arasan 42-S): 75 percent thiram (tetramethylthiuram disulfide) Arasan 75);

50 percent benomvl [methyl 1 - (butyl carbamoyl) - 2 - benzimidazolecarbamatej (Benlate 50 WP):

50 percent captan (N [trichloromethyl thioj - 4 - cvclohexen1, 2-dicarboximide) Captan 50 W): 75 percent captan (N [trichlo romethyl thio]-4- cyclohexene -1. 2-dicarboximide) (Caplan 75):

'All chemicals except Arasan 42-S and Captan 50W were donated by the manufacturers.

35 percent sodium p-(dimethyl amino benzenediazosulfonate) (Dexon 35 WP): and
50 percent trans-1, 2-Bis (n propyIsufonyI) ethylene (Chemagro gche 1843 - Preseed).

The actual rates of application of the fungicides to the seeds of each species are listed in tables 1 and 2.

All fungicides except Arasan 42-S and Captan 50W were applied dry to the seed coats by adding an excess of each fungicide to an Erlenmyer flask containing 150 seeds, shaking. then weighing them to determine the amount of fungicide adhering to them. The application rate was attained by shaking the seeds on a fine wire mesh to remove any excess fungicide.

Arasan 42-S was applied as mixed to the seeds. Captan 50W was applied by soaking them for 24 hours at room temperature in a 0.2 percent solution of the fungicide in water. They were air-dried after treatment. Nontreated seeds served as controls. After treatment. 50 seeds per treatment rate were sown in each of three replicate containers. The containers were placed in two 1965 model E57 Percival growth chambers. Seedlings received 1,800 ± 180 foot-candles of light emitted by eight cool white fluorescent lamps (120 W) and four incandescent (25 W) bulbs. The chambers were regulated to provide a 16-hqur day temperature of 21° C (± 1° C) and an 8-hour night temperature of 12° C (± 1° C). No attempt was made to control relative humidity (range 70-98 percent). Both temperature and relative humidity were monitored continuously throughout the test. All containers were watered to field capacity, at the same rate, two to three times weekly.

TABLE 1.—Effect of seed treatment on survival of Douglas-fir sown in Wind River Nursery soil[1]

Fungicide	Rate of application	Percent of seedlings surviving based on seeds sown
	oz. A.I./100 lbs. seeds	
Arasan 42-S	As mixed	56.0
Arasan 75	1	71.6
	3	70.6
	4	78.5
	35	85.5
Benlate 50	1	60.8
	5	73.0
	6	77.5
Captan 50, soak	2 g/100 ml water	74.7
Captan 75	1	61.3
	2	77.5
	7	67.0
Dexon 35	1	55.6
	3	77.2
	5	83.0
Preseed	10	82.9
Control	None	77.0

[1] Survival measured 54 days after sowing.

TABLE 2.—Effect of seed treatment on survival of ponderosa pine sown in Bend Nursery soil[1]

Fungicide	Rate of application	Percent of seedlings surviving based on seeds sown
	oz. A.I./100 lbs. seeds	
Arasan 42-S	As mixed	4.0
Arasan 75	1	7.3
	2	3.3
	5	22.6
	30	24.2
Benlate 50	1	4.7
	2	2.0
	3	3.3
Captan 50, soak	2 g/100 ml water	0.0
Captan 75	1	3.4
	2	0.7
	3	4.7
Dexon 35	1	30.2
	2	58.6
	4	40.3
Preseed	3	36.9
Control	None	6.7

[1] Survival measured 64 days after sowing.

Data Collection

Data on seedling emergence and mortality were recorded two to three times weekly. Seedlings were counted as emerged when the seed coat or cotyledons no longer contacted the soil. Seedlings cille by root rot were removed as they were tallied. Tissue isolations were made from seedlings periodically to identify causes of mortality. Representative seedlings were immersed in 0.6 percent sodium hypochlorite solution for 1 minute, then rinsed in two changes of sterile distilled water. Five tissue sections from each seedling were placed on *Pythium* and *Fusarium* selective media and incubated for 5 to 10 days at room temperature.

After 9 weeks the test was terminated and the seedlings inspected for damage. Tissue isolations were made from representative seedlings and the soils were assayed for *Pvthium* and *Fusarium spp.*

Results

Soil assay-The Wind River soil had fewer propagules of both fungi at the beginning of the experiment than did the Bend soil; however, at the termination of the test, a greater number of *Pythium spp.* propagules were recorded for the Wind River soil (table 3). In general, numbers of fungi increased in both soils from their initial levels.

Fungicidal seed treatments-The results of the treatments of Douglas.

This article reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

TABLE 3.—Wind River and Bend Nurseries soil assays for Pythium *and* Fusarium *spp.*[1]

Source of soils	*Pythium* sp. propagules/gm		*Fusarium* sp. colonies/plate	
	Initial level	After 9 weeks	Initial level	After 9 weeks
Wind River Nursery	39.6	62.0	13.4	17.6
Bend Nursery	100.4	42.0	38.6	44.6

[1] Soils were collected during March 1973.

fir and ponderosa pine seeds are presented in tables 1 and 2. The data for the optimum treatment rate for three of the best fungicides and the control are presented in figures 1 and 2. Percentage survival of seedlings developing from nontreated seeds was much lower in Bend soil than in Wind River soil, 6.7 and 77.p percent, respectively. Survival of treated ponderosa pine seeds in Bend soil ranged from 0 to 58.6 percent, whereas, survival of Douglas-fir seed in Wind River soil ranged from 55.6 to 85.5 percent. Dexon 35WP applied at a rate of 2 oz. Ami. (active ingredient) per 100 pounds of seeds provided the best protection for ponderosa pine seeds in Bend soil. The best protectant for Douglas-fir seeds was Arasan 75, applied at a rate of 35 oz. A.I. per 100 pounds of seeds. Arasan 42-S, Captan 75, Benlate 50WP, and Captan 50W soak provided little or no protection for either species when compared to nontreated seeds. Emergence of both species was most rapid for Arasan 75 treated seeds.

Tissue isolations-Tissue isolations made from seedlings exhibiting typical early damping-off symptoms predominantly yielded *Pythium spp.* until the fourth week after sowing. Examinations after the fourth week showed *Fusarium* sp. to be most consistently isolated. Affected seedlings exhibited typical late damping-off symptoms: dried foliage, a constriction at the base of the stem. (but seedling remaining erect), and lack of lateral roots (3).

Many seedlings had no tap root, but appeared to have callused below the root collar area and produced new lateral roots. These seedlings were often stunted.

Of the 128 ponderosa pine seedlings inspected at the termination of the experiment, 66 percent exhibited root lesions from which *Fusarium spp.* were isolated. Ad. ditional isolations from damaged ponderosa pine seedling stems yielded *Fusarium spp.* 55.8 percent of the time and *Pythium spp.* 6.0 percent. *Fusarium spp.* were isolated from damaged trees in all treatments. Tissue isolations were not attempted from Douglas-fir seedlings at the ter mination of the experiment because little or no damage was noted.

Discussion and Conclusions

The seed treatments, particularly Arasan 75. Dexon 35WP, and Preseed. provided some protection for Douglas-fir and ponderosa pine against early damping-off, but provided little or no protection against later stages of root rot caused by *Fusarium* spp. Approximately 3 weeks after sowing the seeds, survival of merged seedlings decreased in response to *Fusarium* root rot. Losses continued until the sixth week, after which little mortality occurred.

A number of treatments provided little or no protection when compared to controls. A possible explanation for this anomaly is that these fungicides may have been phytotoxic at the levels tested.

Losses of seedlings were much greater in the Bend soil than in the Wind River soil. These differences can he explained by the differences in numbers of fungi detected in the soil assay. The Bend Nursery has had a history of extensive seedling losses to *Fusarium* root rot (2.5). but the Wind River Nursery has experienced only occasional losses.

All the work reported here was conducted under controlled laboratory conditions. The results indicate that field trials with Dexon 35WP, Arasan 75, and Preseed as seed treatments are warranted. Soil drenching with Dexon 35WP after sowing seeds may be useful to reduce losses to *Fusarium* root rot.

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