FUSARIUM ROOT DISEASE OF CONTAINERIZED SEEDLINGS AT THE MONTANA STATE NURSERY, MISSOULA

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Growers at the Montana State Nursery in Missoula were recently concerned about mortality of containerized Douglas-fir (Pseudotsugae menziesii (Mirb.) Franco) seedlings which were several months old. Mortality was most noticeable after seedlings were removed from the greenhouse and placed outside under shade. Affected seedlings were scattered randomly, although certain seedlots appeared more affected than others.

METHODS

Samples of necrotic and declining seedlings were collected for laboratory analysis and isolation of associated fungi. Root systems of dead and declining seedlings were mostly necrotic. Few lateral feeder roots were intact, and extensive cortical decay and watersoaking was evident. Epidermal tissues were easily detached from necrotic roots.

Several isolations from necrotic root tissues were made on standard water agar with emerging fungi transferred to and maintained on potato dextrose agar (PDA) slants. Because we felt that Fusarium spp., common pathogens of conifer seedlings, may be associated with the mortality, portions of necrotic root tissues were also placed on a selective medium for Fusarium (described by Nash and Snyder (1962).

Samples of Douglas-fir seed from a representative seedlot were obtained and assayed for mycoflora including possible contamination with Fusarium. Seed underwent nine treatments (table 1) before being incubated on either selective Fusarium medium or water agar at 24° C under continuous cool fluorescent light for 6-12 days. Each treatment consisted of 10 seed replicated five times. Seed were examined periodically for germination until all seed had germinated or until seed had incubated for 12 days. Seed were considered germinated if their hypocotyls had extended outside the seedcoat. Seed were also designated as having their seedcoat broken open, which would probably indicate an early stage of germination. Fungi on seed were identified to genus except for Fusarium, for which species were determined using the taxonomic scheme of Snyder and Hansen (1940). Bacteria were also noted on seed but were not identified.

Table 1.--Treatments to evaluate mycofloral populations and germination of Douglas-fir seed from the Montana State Nursery, Missoula.

No.	Treatment
1	None - seed placed directly on selective Fusarium medium
2	Seed soaked in standard tap water for 24 hours and then placed directly on selective Fusarium medium.
3	Seed rinsed in continuously running tap water for 48 hours and then placed directly on selective Fusarium medium.
4	Seed soaked in a 5.25 percent aqueous sodium hypochlorite solution for 2 hours and then placed directly on selective

Fusarium medium.

5 Seed soaked in standard tap water for 24 hours, dusted with captan[1] at the rate of 15.3 g of fungicide per 100 g of seed, and then placed directly on selective Fusarium medium.

6 Seed rinsed in continuously running tap water for 48 hours, aseptically dissected to expose inner seedcoat and endosperm and then placed directly on selective Fusarium medium.

7 Seed soaked in a 3 percent hydrogen peroxide solution for 24 hours and then placed directly on selective Fusarium medium.

8 Seed soaked in a 3 percent hydrogen peroxide solution for 64 hours and then placed directly on selective Fusarium medium.

9 Seed soaked in standard tap water for 24 hours and then placed directly on water agar.

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[1]Captan = N[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide

RESULTS

Isolations - Two species of Fusarium were consistently isolated from necrotic roots of dead and dying Douglas-fir seedlings. Isolation of Fusarium was much more common on Fusarium selective media than on water agar. On water agar, rapid superficial growth of saprophytic fungi, especially Penicillium and Alternaria, often obscured presence of other fungi, including Fusarium.

Fusarium oxysporum Schlecht. was isolated from several seedlings. At least two distinct isolates of this species were detected. Their differences were related to extent of sporodochial and macroconidial production in culture. However, both had characteristic chlamydospores (figure 1) and small, mostly unbranched microconidiophores (figure 2) which are definitive for this species (Booth 1975).

The other species of Fusarium commonly isolated was F. solani (Mart.) Sacc. This species produced characteristic thick-walled macroconidia that were widest in their upper half (figure 3). The fungus also produced elaborate, branched microconidiophores and extensive thick-walled chlamydospores (figure 4) in culture.

Figure 1.--Fusarium oxysporum isolated from nectoric roots of Douglas-fir seedlings. Chlamydospores (resting structures) produced in culture (X450).

Figure 2.--Fusarium oxysporum isolated from necrotic roots of Douglas-fir seedlings. Microconidiophores which are small with few branches (X450).

Figure 3.--Fusarium solani isolated from necrotic roots of Douglas-fir seedlings. Macroconidia which are generally widest in their upper half (black arrow) and microconidia (red arrow) (X450).

Figure 4.--Fusarium solani isolated from necrotic roots of Douglas-fir seedlings. Chlamydospores (resting structure) produced in culture (X450).

from the seedlings.

Seed Germination - Effects of the treatments on Douglas-fir seed germination are summarized in table 2. All treatments reduced germination over the 12-day incubation period as compared with untreated seed (treatment 1). However, hydrogen peroxide treatments (#7 and #8) initially stimulated germination. Bleach (sodium hypochlorite - treatment 4) and captan dust (treatment 5) greatly reduced germination, which may have been due to phytotoxic effects.

Table 2.--Effects of selected treatments on Douglas-fir seed germination.

	Percentage of Seed[1]					
Treatment[2]	Germinated	Seedcoat open	Not germinated	Germinated	Seedcoat open	Not germinated
1	20	12	68	78	4	18
2	8	8	84	12	10	78
3	8	4	90	24	6	70
4	0	18	82	6	12	82
5	0	8	92	2	8	90
7	32	32	36	40	26	34
8	66	22	12	72	22	6
9	40	6	54	58	2	40

[1]Germinated seed were those with hypocotyl extension outside the seedcoat. Those seed classified as seedcoat open had a noticeable break in the seedcoat, but no hypocotyl extension. Nongerminated seed showed no evidence of seedcoat breakage or hypocotyl emergence.

[2]Treatments are described in Table 1. Treatment 6 involved dissecting seed before incubation; therefore germination values are not possible for this treatment.

Seed Fungi - Occurrence of fungi on Douglas-fir seed by treatment is outlined in table 3. Fusarium was isolated from untreated seed and that which was soaked in water or dissected. The two species found were F. oxysporum and F. solani. Isolates of both fungi were very similar to those obtained from necrotic seedlings. Fusarium isolated from dissected seed were growing on the inner portion of seedcoats.

Table 3.--Effects of selected treatments on mycoflora of Douglas-fir seed.

Treatment[1]	Percent of Seed Colonized with Fungi									All treatment
	1	2	3	4	5	6	7	8	9	
Fusarium	4	-	2	-	-	4	-	-	-	1.1
Pythium	2	2	2	-	-	6	-	4	-	
Rhizoctonia	-	-	-	-	-	-	-	-	-	
Penicillium	86	64	82	28	-	72	10	-	52	
Aureobasidium	32	4	6	-	-	44	82	98	16	
Cladosporium	10	4	8	2	-	12	-	-	4	
Phoma	6	-	-	-	-	-	-	-	4	
Aspergillus	-	-	-	-	-	-	-	-	2	
Alternaria	-	-	-	-	-	-	-	-	30	
Rhizopus	-	-	-	-	-	-	-	-	4	
Mucor	-	-	-	-	-	-	-	-	4	
Trichoderma	2	-	6	-	-	-	4	-	28	
Chaetomium	-	-	-	-	-	-	-	-	2	0.2
Unidentified bacteria	8	98	68	2	100	50	-	-	98	47.1

[1]Treatments are described in Table 1.

Many other genera of fungi were isolated from Douglas-fir seed (table 3). Several of these are potentially pathogenic, the most important being Pythium, Rhizoctonia, Cladosporium, and Phoma. Pathogenicity tests are required to determine the potential for all these fungi, including Fusarium, to cause seed decay and mortality of young seedlings.

CONCLUSIONS

1. Two species of Fusarium, F. oxysporum and F. solani, were consistently isolated from necrotic roots of Douglas-fir seedlings and found on and within seed of a representative seedlot from which the seedlings were grown. Although inoculation tests are required to confirm pathogenicity of these isolates, it is suspected that either or both species are responsible for seedling mortality. Past experience with these fungi indicate that they are common pathogens of conifer seedlings, although they usually cause more problems on bareroot stock. Fusarium oxysporum may invade healthy seedlings when they are young and can initiate disease then or later as the seedlings grow. Apparently,

environmental stress factors, host susceptibility, and inherent virulence of the fungus are all involved in disease expression. Fusarium solani causes cortical rot of roots and has been shown to initiate disease on several species of conifer seedlings. Both fungal species are commonly seedborne, as verified in this test. Their occurrence on seed is especially more common when cones are collected from the ground or squirrel caches than when collected directly from trees.

2. Douglas-fir seed germination is affected by common seed treatments used to reduce fungal contamination. In this test, 3 percent hydrogen peroxide reduced germination least while removing most fungi from seed. Captan and sodium hypochlorite (bleach) both reduced fungal contamination, but also greatly reduced germination. Soaking or rinsing seed with water will reduce surface contamination, but will not eliminate all fungi on seed. From this limited trial, it appears that hydrogen peroxide provided the most satisfactory seed treatment of those tested.

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