OCCURRENCE OF FUSARIUM ON DOUGLAS-FIR SEED

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FROM THE COEUR D'ALENE NURSERY

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An outbreak of disease on Douglas-fir (<u>Pseudotsuga menziesii</u> (Mirb.) Franco) seedlings occurred during 1982 at the Colorado Hydroponics container nursery in Lyons, Colorado. The disease was investigated by Dr. L. R. Fuller (R-2 FPM) and determined to be caused by <u>Fusarium</u> spp., common pathogens of conifer seedlings. Losses from this disease approached 100 percent. Growers were concerned that the disease may have been initiated by pathogens introduced on contaminated seed. <u>Fusarium</u> may be seedborne and is often not removed by standard water treatments of seed that are normally performed before sowing. Therefore, to evaluate if contaminated seed may have been involved, samples of selected seedlots were assayed for presence of <u>Fusarium</u>.

Tests were initially conducted by Dr. Fuller on five Douglas-fir seedlots. He found <u>Fusarium</u> on only two of the seedlots (B and C) with infection rates of 1.3 and 6.1 percent, respectively. Samples from these same seedlots were reevaluated for occurrence of <u>Fusarium</u> and results are reported here.

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METHODS

Five Douglas-fir seedlots (designated A-E) were supplied by J. F. Myers (Coeur d'Alene Nursery) for determining occurrence of <u>Fusarium</u> directly on or within seed. In the first test, seed from each lot underwent three treatments. Ten seed from each lot were placed directly on selective <u>Fusarium</u> medium² without prior soaking or other treatment. Likewise, 10 seeds were placed on the selective media after being washed in a continuous tap water rinse for 2 hours. Twenty-five seeds per lot were also treated with the running water rinse, but were then aseptically dissected to expose the inside of their seedcoat and endosperm before being placed on the selective media.

In a second test, the same five seedlots were assayed for <u>Fusarium</u> after being stored at 5° C for <u>6</u> months. In this test, 20 seeds from each seedlot were placed directly on selective media without prior treatment. All seeds were incubated on selective media at 24° C in alternating 12-hour cycles of light and dark for 5-7 days. <u>Fusarium</u> infection was characterized by active growth of sporulating fungal colonies on the seed and over the medium.

² Selective medium described by Nash, S. M. and W. C. Snyder, 1962. Quantitative estimations by plate counts of propagules of the bean root rot <u>Fusarium</u> in field soils. Phytopathology 52(6): 567-572.

RESULTS

Results of both tests are summarized in Table 1. Fusarium was obtained from all five seedlots, although levels of infection were generally low. Fusarium was located both on and within seed. Rinsing with water did not completely remove Fusarium from seedcoats. Also several of the dissected seed contained Fusarium inside the seedcoat or on the endosperm. Also, Fusarium persisted on seed stored at low temperatures for several months.

Table 1.--Occurrence of <u>Fusarium</u> spp. on Douglas-fir seed from the Coeur d'Alene Nursery, Idaho.¹

	Test 1 Treatment			Test 2
Seedlot	None	Rinsed	Rinsed & Dissected	No treatment
А	1	0	0	0
В	1	1	2	2
С	1	0	2	5 /
D	1	0	3	0
E	0	0	1	0
ALL	4	/ 1	1.	7
SEEDLOTS	(8.0)	(2.0)	(6.4)	(7.0)

¹ Figures in table are number of seed from which <u>Fusarium</u> spp. were obtained. Percentages are in parentheses. Three species of <u>Fusarium</u> were identified on Douglas-fir seed.⁴ The most common was <u>F</u>. <u>oxysporum</u> Schlect. This species was characterized by typical thin-walled falcate macroconidia, abundant microcondia, and distinct single or double thick-walled chlamydospores. Three distinct isolates of <u>F</u>. <u>oxysporum</u> were identified on the basis of growth habit in culture (on potato dextrose agar slants) and ratio of microconidia to macroconidia produced in culture. The first isolate (82-59A) produced a greyish-white appressed mycelium, few sporodochia, and distinct violet pigment in culture. This isolate also produced many macroconidia of varying lengths and septations and small ovate to circular microconidia (figure 1). Chlamydospores produced by this isolate were typical for the species as a whole and abundant in cultures older than 14 days (figure 2).

The second isolate of <u>E</u>. <u>oxysporum</u> (82-59B) produced whitish appressed mycelium, abundant salmon colored sporodochia, and localized violet pigment in culture. This isolate produced few macroconidia and abundant large, sometimes septate, microconidia in culture (figure 3). Chlamydospores from this isolate were similar to those of other isolates (figure 4).

⁴ Species of <u>Fusarium</u> were identified using the taxonomic scheme outlined by Tousson, T. A. and P. E. Nelson. 1968. A pictorial guide to the identification of <u>Fusarium</u> species according to the taxonomic system of Snyder and Hansen. Pennsylvania State Univ. Press, University Park. 51 pp.

The third isolate of <u>F. oxysporum</u> (82-59F) produced a whitish floccose mycelium over a violet-colored colony with abundant salmon-colored sporodochia. This isolate produced abundant macroconidia and many small to ovate microconidia (figure 5).

Another <u>Fusarium</u> species isolated from seed was <u>F. solani</u> (Mart.) Sacc. This species was characterized by production of thick-walled macroconidia which were generally widest in their upper half, microconidia of various shapes and sizes, and individual terminal or intercalary chlamydospores. The isolate produced abundant macroconidia, but few microconidia (figure 6). This species was much less common than <u>F. oxysporum</u> on seed.

The third species isolated was <u>F</u>. <u>roseum</u> (Lk.) Sacc. This fungus produced characteristic red pigment, salmon colored sporodochia, abundant, narrow macroconidia (figure 7), no microconidia, and few chlamydosores in culture.

CONGLUSIONS

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- Although <u>Fusarium</u> was found on all tested Douglas-fir seedlots, low levels of infection would preclude extensive disease incidence from contaminated seed alone. Apparently, other sources of inoculum were available for the rapid buildup and spread of the disease that occurred.
- 2. <u>Fusarium</u> was isolated from both on and within seed. Washing or soaking seed in water will probably not remove all inoculum, even though numbers of propagules may be reduced. Low level of infection within seed would likely result in scattered, isolated seedling disease.

3. The three species of <u>Fusarium</u> isolated from Douglas-fir seed were <u>F</u>. <u>oxysporum</u>, <u>F</u>. <u>solani</u>, and <u>F</u>. <u>roseum</u>. <u>Fusarium oxysporum</u> was by far the most common. <u>Fusarium moniliforme</u> was not isolated, even though this species was commonly obtained from diseased seedlings by Dr. Fuller. There may have been other sources of inoculum for this species. Pathogenicity tests were not conducted on the <u>Fusarium</u> isolates found on seed. Since there are both pathogenic and saprophytic strains of all three species, role of these fungi in causing seedling diseases in unknown.

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Figure 1.--Fusarium oxysporum (Isolate 82-59A). Photomicrograph of macroconidia and microconidia in culture (X450).



Figure 2.--<u>Fusarium oxysporum</u> (Isolate 82-59A). Photomicrograph of chlamydospores in culture (X450).



Figure 3.--Fusarium oxysporum (Isolate 82-59B). Photomicrograph of microconidia (abundant) macroconidia (rare) in culture (X450).



Figure 4.---Fusarium oxysporum (Isolate 82-59B). Photomicrograph of chlamydospores in culture (X450).



Figure 5.--Fusarium oxysporum (Isolate 82-59F). Photomicrograph of macroconidia and microconidia in culture (X450).



Figure 6.--Fusarium solani. Photomicrograph of abundant macroconidia and few microconidia in culture (X450).



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Figure 7.--<u>Fusarium roseum</u>. Photomicrograph of macroconidia in culture (X450).