Diseases of Douglas-Fir Seedlings Caused by Fusarium oxysporum

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ABSTRACT

Isolates of *Fusarium oxysporum* were obtained from roots of Douglas-fir seedlings without disease symptoms or with one of the following diseases: postemergence damping-off, root rot, or corky root. Isolates varied in infectivity, pathogenicity, types of disease they caused, and their ability to kill seedlings of different ages. Pathogenicity was greatest in seedlings growing in nutrient agar, intermediate in sterile nursery soil, and lacking in sterile peat-sand mixture. Phytopathology 61:467-470.

Diseases of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings in coastal British Columbia are often associated with infection by *Fusarium oxysporum* Schlecht. This fungus was associated nearly always with pre-emergence damping-off (6), and to a lesser extent with postemergence damping-off (J. E. Bier, *personal communication*). It was almost invariably associated with "top-blight", a needle infection (D. C. Buckland, *personal communication*), and with root rot in which the phloem and cortex of the roots are destroyed (3, 5, 10). *Fusarium oxysporum* and *Cylindrocarpon destructans* were isolated with equal frequency from seedlings with corky root disease, which features deformed roots and stunted shoots (5). On the other hand, *F. oxysporum* was also isolated from the roots of over 80% of healthy seedlings growing in the same plots as root-rotted seedlings (4).

Although *F. oxysporum* caused damping-off in artificial inoculation tests (3, 12), the fungus has not been proven to be a primary cause of root rot. Nor has it been shown that the isolates from either disease can cause the other, or that isolates from healthy seedlings are potentially pathogenic.

Postemergence damping-off was influenced by soil composition, especially some types of organic matter (J. E. Bier and D. C. Buckland, *personal communications*), and appeared to be confined to seedlings in the cotyledonary stage, whereas root rot (11) and corky root (5) occurred only in older seedlings. Experiments were therefore carried out to ascertain the importance of these fungus, soil, and seedling factors on diseases of Douglas-fir seedlings.

MATERIALS AND METHODS.—Containers were made from quart milk bottles cut into 5-inch-long tops and 5.5-inch-long bases. Granite chips were placed 1 inch deep in each base to provide drainage; soil was then gently firmed into the base to within 0.75 inch of the lip. The bottle top was fitted to the base with a wide rubber band and the mouth capped with polyethylene.

California soil mix, 50:50 peat:sand, pH 6.5, with inorganic fertilizer was moistened to 60% field capacity. The mix was modified by using a coarser sand than specified (2). Bottles containing this soil mix were steamed 1 hr at 15 psi. This mix does not become phytotoxic when steamed (2). Forest nursery topsoil, silt loam, pH 5.6, was thoroughly mixed with an equal volume of washed coarse sand to reduce compaction, and moistened to 60% field capacity. Because field soil may become phytotoxic when steamed (9), bottles containing this soil were fumigated in polyethylene bags with methyl bromide at a dosage of 100 g/3,000 g soil. After 48 hr fumigation, the bases were removed from the bags and aired for 1 week. Absence of residual methyl bromide toxicity in the soil to *F. oxysporum* was verified by the agar slide technique (7), and absence of toxicity to Douglas fir by germination tests. Soil sterility was verified by inserting agar-coated slides in the soil, and by incorporating soil in cooled melted malt extract agar.

Douglas-fir seeds from a high viability lot (germination 80%) were stratified by soaking in tap water for 24 hr, then surface-dried and stored at 2 C for 21 days in a loosely closed jar. The seeds were surface-sterilized by immersion in 70% ethyl alcohol for 5 sec and 0.1% mercuric chloride for 3 min, then rinsed in three changes of sterile water. Five seeds were spaced equally on the soil surface in each bottle, then covered with 0.25-inch washed sterile sand.

Twenty-five ml of nutrient medium were pipetted into culture tubes. The medium contained agar, 10 g/1; $MgSO_4 \cdot 7H_2O$, 0.1 M; $MgCl_2 \cdot 6H_2O$, 1.5 M; KCl, 1.0 M; KH_2PO_4, 1.0 M; $Ca(NO_3)_2 \cdot 4H_2O$, 2.0 M; microelements (1 ml/liter H_3BO_3, $ZnCl_2$, $Na_2MoO_4 \cdot 2H_2O$, iron chelate). The pH was adjusted to 5.0. Iron chelate was added after autoclaving. Unstratified seeds were disinfected by immersion in 35% hydrogen peroxide for 0.5 hr. They were placed on malt extract agar for 1 week to detect contamination; then an uncontaminated germinant seedling was planted in each culture tube and the tube was capped with polyethylene.

Fusarium oxysporum (identified by Dr. C. Booth, Commonwealth Mycological Institute, Kew, England) was isolated from the following Douglas-fir sources: (i) fresh postemergence damped-off seedlings (PEDO); (ii) the roots of healthy seedlings about 8 weeks old, after damping-off had ceased (PEDO survivors); (iii) the roots of root-rotted 1-0 seedlings (root rot); (iv) the roots of healthy 2-0 seedlings (root rot survivors); (v) The roots of 2-0 seedlings with corky-root symptoms (corky root).

Five isolates from each source were cultured on duplicate petri plates containing soil extract agar (8). When macrospores and chlamydospores had developed, inoculum was prepared by homogenizing the contents of

the two plates with 250 ml distilled water in a blender at slow speed for 3 min. The inoculum was allowed to stand for 3 days to increase chlamydospore formation (1). Spores were counted in 10 samples of each inoculum. Macrospore and chlamydospore concentrations averaged 30,000 and 29,000/ml, respectively, for all inocula.

Ten ml of each inoculum were evenly pipetted over the soil surface in the bottles (i) before the seeds were covered with sand; or (ii) after the seedlings had produced secondary needles. In addition, inocula from either two or four sources (Table 1) were combined in equal volumes, and 10 ml of each mixture were pipetted over the soil after seedlings had produced secondary needles. Viability of the inoculum was verified on malt extract agar. Water containing homogenized soil extract agar discs was pipetted over the soil in controls.

Seedlings growing in culture tubes were inoculated by placing 0.1 ml of the above inocula on the surface of the nutrient agar (i) 7 days after the seedlings had been transplanted; or (ii) after they had produced secondary needles.

The experiment was conducted in a partially controlled environment based on average nursery conditions. Bottles and culture tubes were placed in Wisconsin tanks. Soil temp was maintained at 15.5 C for 1 week, raised 1.1 each week for the following 5 weeks, then maintained at 21 C. Ambient temperature in the bottles ranged from 21 to 32 C, averaging 28 C. Very high output, cool-white fluorescent, and about 10% incandescent light sources supplemented greenhouse daylight for 14 hr each day for 12 weeks; the intensity was then halved, and day length reduced to 10 hr. Simultaneously, soil temperature was reduced to 15.5 C over a 5-week period.

To maintain seedling growth, 50 ml nutrient solution were added to each bottle containing California soil mix 10 weeks after seeding, and on three occasions, 25 ml water were added to those containing nursery soil.

Seedlings were removed for examination after 5 months, then measured and weighed. Tissues from roots of killed or surviving seedlings were surface-sterilized in sodium hypochlorite solution containing 0.5% available chlorine for 5 min. They were then placed on 2% malt extract agar. Isolations were made from a 5-mm section taken from the taproot of surviving seedlings and from the margin of diseased tissues in killed seedlings.

The experimental design in the nursery soil experiment consisted of five randomized complete blocks, each containing five single inoculum treatments applied at 2 times and two combined inoculum treatments applied once, plus controls, or a total of 70 bottles. The design of the California soil mix experiment consisted of six blocks, each containing four single inoculum treatments applied at two times and five combined inoculum treatments applied once, plus controls, or a total of 90 bottles. Data were analyzed in original or $\sqrt{k+1}$ transformation. Tables show the original data. Differences cited were significant ($P = .05$ unless otherwise stated) by Duncan's multiple range test.

RESULTS AND DISCUSSION.—None of the inocula added to the soil significantly reduced seedling emergence either in the nursery soil or in the California soil mix.

In nursery soil, damping-off was influenced mostly by the stage of seedling development (Table 2). Significant damping-off resulted from addition to the soil of all inocula except PEDO survivors and corky root. Addition of combined inocula also resulted in significant damping-off. Difference in damping-off between the two combinations indicated possible interactions between constituent isolates.

Root rot was influenced by an interaction between seedling growth stage and inoculum source (Table 2). Adding PEDO inoculum to the soil resulted in little root rot. By contrast, severe root rot occurred after

TABLE 1. Sources of combined *Fusarium oxysporum* inocula used to inoculate Douglas-fir seedlings

Inoculum combination	Sources of constituent isolates (from Douglas fir)
A	PEDO[a], survivors of PEDO, root rot, corky root
B	PEDO, root rot, corky root, survivors of root rot
C	Survivors of PEDO, root rot
D	PEDO, survivors of PEDO
E	PEDO, root rot
F	Root rot, survivors of root rot
G	PEDO, survivors of root rot

[a] Postemergence damping-off.

TABLE 2. Postemergence damping-off (PEDO) and root rot in Douglas fir in nursery soil following inoculation at 2 times with *Fusarium oxysporum* from different sources

	PEDO		Root rot	
	Time of inoculation in relation to seedling stage			
Source of inoculum	At sowing	Post-cotyledon	At sowing	Post-cotyledon
	%	%	%	%
PEDO	27.0 ab[c]	6.7 c	14.0 c	14.0 c
Survivors of PEDO	12.3 c	18.7 c	33.2 a	16.0 c
Root rot	36.8 a	11.3 c	40.0 a	12.3 c
Survivors of root rot	33.9 a	14.7 c	24.0 b	20.3 b
Corky root	6.7 c	0 d	26.6 b	50.9 a
Combination[a] A	37.5 a		29.2 ab	
Combination B	21.2 bc		24.2 b	
Control	16.3 bc[b]	0 d	4.0 d[b]	13.0 c[b]

[a] Combinations A and B contained all inoculum sources (Table 1) except survivors of root rot and survivors of PEDO, respectively.

[b] *Fusarium oxysporum* not reisolated from these seedlings.

[c] Means followed by different letters are significantly different ($P = .05$).

the addition of corky root or root rot survivor inocula, or combined inocula. Adding root rot or PEDO survivor inoculum to the soil at sowing resulted in severe root rot, but adding it at the postcotyledonary stage had no significant effect.

No seedlings developed symptoms of corky-root disease. The relative severity of damping-off and root rot indicated that pathogenicity of *F. oxysporum* from PEDO source decreased in older seedlings, whereas the pathogenicity of *F. oxysporum* from corky-root source increased. Pathogenicity of the fungus from root rot source appeared to depend on infection at an early seedling stage even though root rot symptoms were not manifested until later. The pathogenicity of isolates from PEDO survivor source was apparently limited to young seedlings and to causing root rot.

TABLE 3. Per cent root infections in Douglas-fir seedlings surviving inoculation with *Fusarium oxysporum* from different sources at different times, in two soil types

	Nursery soil		California mix	
	Time of inoculation in relation to seedling stage			
Source of inoculum	At sowing	Post-cotyledon	At sowing	Post-cotyledon
PEDO[a]	100 a[b]	100 a	27 b	21 c
Survivors of PEDO	80 a	80 a	54 a	17 c
Root rot	100 a	60 b ($P = .15$)	32 b	14 c
Survivors of root rot	75 ab	60 b ($P = .15$)	34 b	6 d
Corky root	100 a	40 c		
Combination[c] A	100 a			
Combination B	100 a			
Combination C (Survivors of PEDO & root rot)				34 b
Combination D (PEDO & survivors of PEDO)				38 b
Combination E (PEDO & root rot)				43 b
Combination F (root rot & survivors of root rot)				25 bc
Combination G (PEDO & survivors of root rot)				24 bc
Controls	0 d	0 d	6 d[d]	0 d

[a] Postemergence damping-off.
[b] Means followed by different letters are significantly different ($P < .05$) unless otherwise indicated.
[c] Combinations A and B contain all inoculum sources except survivors of root rot and survivors of PEDO, respectively (Table 1).
[d] Presumably of seed-borne origin.

Fusarium oxysporum was reisolated from more than 90% of diseased seedlings. Losses in controls were associated with *Penicillium* sp., presumably a seed-borne facultative parasite of weak seedlings.

In California mix, none of the *F. oxysporum* inocula caused more than 1% damping-off or root rot. This result is consistent with the successful use of peat to reduce disease of Douglas fir seedlings in nursery beds, supposedly due to the acidity of peat (J. E. Bier and D. C. Buckland, *personal communications*). P. J. Salisbury (*personal communication*), however, found that *F. oxysporum* isolates from diseased seedlings were tolerant of a wide pH range.

Generally, the percentage of nonpathogenic infection was greater when inoculation was done at sowing than at the postcotyledonary stage (Table 3), indicating a host-age effect. The almost complete infection of seedlings inoculated in nursery soil at sowing excludes infection frequency as a factor in disease development in young germinants. However, lower infection frequencies from some inocula in postcotyledonary seedlings may account in part for the lower disease incidence at this stage. Infections were fewer in California mix than in nursery soil, indicating a soil effect. Shoot wt per seedling was greater in California mix (131 mg) than in nursery soil (37 mg) when noninoculated seedlings of the same age were compared. This increased vigor, presumably due to the more favorable physical and chemical properties of California mix, may be correlated with seedling resistance to infection by *F. oxysporum*. The almost complete absence of disease in this medium was obviously not due to lack of infection.

The proportion of infections that developed disease symptoms varied greatly among inocula (Table 4), indicating differences in virulence, and also varied between seedling growth stages, indicating a difference in postinfection resistance. This resistance was greater in older seedlings, except to the corky-root inoculum.

In culture tubes containing nutrient agar, all inocula had approx the same pathogenic potential, sensu Vaartaja & Cram (13), except PEDO, which was weakly

TABLE 4. Ratio of total disease[a] to total infection[b] in Douglas-fir seedlings inoculated with 5 sources of *Fusarium oxysporum* in nursery soil

	Time of inoculation in relation to seedling stage	
Source of inoculum	At sowing	Post-cotyledon
PEDO[c]	0.41	0.21
Survivors of PEDO	0.51	0.40
Root rot	0.77	0.34
Survivors of root rot	0.65	0.47
Corky root	0.33	0.72
Combination[d] A	0.67	
Combination B	0.45	

[a] Total disease per cent = postemergence damping-off per cent + root rot per cent.
[b] Total infection per cent = total disease per cent + per cent root infection of surviving seedlings.
[c] Postemergence damping-off.
[d] Combinations A and B (Table 1) contain all inoculum sources except survivors of root rot and survivors of PEDO, respectively.

TABLE 5. Per cent seedlings killed and number of days in Douglas-fir seedlings survived after inoculation with *Fusarium oxysporum* from different sources at 2 different times in nutrient agar

Source of inoculum	% Killed		Avg. no. days survived	
	Time of inoculation in relation to seedling growth			
	Germination	Postcotyledon	Germination	Postcotyledon
PEDO[a]	100 a[b]	29 b	33 a	21 b
Survivors of PEDO	100 a	100 a	31 a	18 b
Root rot	100 a	100 a	17 c	22 bc
Root rot survivors	100 a	86 a	19 c	21 bc
Corky root	100 a	71 a	34 a	27 ab
Control	0 c	0 c	150+ d	150+ d

[a] Postemergence damping-off.
[b] Means followed by different letters are significantly different ($P = .05$).

virulent to postcotyledon seedlings (Table 5). Aggressiveness, indicated by the average number of seedling survival days, varied widely among inocula.

It is unlikely that chance would account for selection of five similar isolates from each substrate type. Differences among inocula, therefore, indicated differences of strain; viz., in infectivity, virulence, aggressiveness, and response to host age and to soil factors. The pathological stability of each isolate cannot be assumed from the evidence so far; however, some tentative classification can be made: (i) Strains of *F. oxysporum* causing postemergence damping-off probably cause little other disease; thus, it is unlikely that the damped-off seedlings would provide inoculum to infect nearby postcotyledonary seedlings. Experience supports this assumption. (ii) Strains of *F. oxysporum* persisting in seedlings that survived damping-off probably become active later to cause root rot; probably some change within the host triggers activity. Certainly the lack of a clear-cut relationship between root rot and specific environmental factors in the nursery indicates that internal rather than external factors are responsible for pathogenicity. (iii) Similarities between isolates of *F. oxysporum* root rot and root rot survivor in infectivity, pathogenicity, and aggressiveness suggest that they are one strain. The very high correlation in the incidence of root rot and nonpathogenic isolates in the same nursery plots (5) justifies this grouping. Although inocula from both isolates caused significant damping-off in the experiments, they do not do so in the nursery, as implied by the relative inability of inoculum from damped-off seedlings to cause root rot. In the normal course of nursery operations, young seedlings in the cotyledon stage would not be exposed to root rot inoculum because root-rotted seedlings from the previous crop have completely decomposed in the soil by the time the new crop is sown. (iv) Corky-root isolate appears indifferent to host age for infection, but pathogenicity is expreseed only in older seedlings, an observation frequently made in the nursery.

LITERATURE CITED

1. ALEXANDER, J. V., J. A. BOURRET, A. H. GOLD, & W. C. SNYDER. 1966. Induction of chlamydospore formation by Fusarium solani in sterile soil extracts. Phytopathology 56:353-354.
2. BAKER, K. F. 1957. [ed.]. The U.C. system for producing healthy container grown plants. Calif. Agr. Exp. Sta. Manual 23.
3. BLOOMBERG, W. J. 1965. The effect of chemical sterilization on the fungus population of soil in relation to root disease of Douglas-fir seedlings. Forest. Chron. 41:182-187.
4. BLOOMBERG, W. J. 1966. The occurrence of endophytic fungi in Douglas-fir seedlings and seeds. Can. J. Bot. 44:413-420.
5. BLOOMBERG, W. J., & W. R. ORCHARD. 1969. Chemical control of root disease of Douglas-fir seedlings in relation to fungus and nematode populations. Ann. Appl. Biol. 64:239-244.
6. BLOOMBERG, W. J., & J. TRELAWNY. 1970. Effect of thiram on germination of Douglas fir seeds. Phytopathology 60:1111-1116.
7. CHINN, S. H. F. 1953. A slide technique for the study of fungi and actinomycetes in the soil with special reference to Helminthosporium sativum. Can. J. Bot. 31:718-724.
8. LOCHHEAD, A. G. 1940. Quantitative studies of soil micro-organisms. III. Influence of plant growth on the character of the bacterial flora. Can. J. Res. 18: 42-53.
9. NEWHALL, A. G. 1955. Disinfestation of soil by heat, flooding and fumigation. Bot. Rev. 21:189-250.
10. SALISBURY, P. J. 1954. A review of damping-off of Douglas-fir seedlings in British Columbia. Forest Chron. 30:407-410.
11. SHEA, K. R., & J. H. REDISKE. 1961. Pathological aspects of germination and survival of Douglas-fir in controlled environment. Weyerhaeuser Co. Forest Res. Note 41.
12. TINT, H. 1945. Studies in the Fusarium damping-off of conifers. I. The comparative virulence of certain Fusaria. Phytopathology 35:421-439.
13. VAARTAJA, O., & W. H. CRAM. 1956. Damping-off pathogens of conifers and of caragana in Saskatchewan. Phytopathology 46:391-397.