Fusarium Root Rot of Douglas-Fir Seedlings

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The assistance of W. Lock is gratefully acknowledged.

Accepted for publication 3 October 1972.

ABSTRACT

Incidence of root rot disease of Douglas-fir seedlings caused by *Fusarium oxysporum* varied significantly among seedling provenances, sampling years, nurseries, and seedbeds within nurseries. It was greater on the north side of beds with sideboards than on the south side, and in spring-sown open beds than in fall-sown closed beds. It was not correlated with seed germinability, seedling stand density, or seedling size and weight. The disease was most prevalent in seedlings transplanted into infested soil up to 30 days after sowing, and was virtually absent in older transplants; symptoms did not occur until several months after transplanting. Disease incidence was greatly increased by temperatures exceeding 23 C for more than 6 hr/day during the 1st month after sowing. However, temperature had no effect in soil containing high *Streptomyces* populations. Single seedling infections significantly outnumbered group infections. The disease occurred from May to February, peaking in late August. It was distributed randomly within beds.

Phytopathology 63:337-341

Additional key words: Pseudotsuga menziesii.

Root rot caused by *Fusarium oxysporum* Schlecht. is an economically important disease of 1-year-old seedlings of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] in British Columbia nurseries (6). It is distinct in symptoms and time of occurrence from other diseases of Douglas fir caused

by *F. oxysporum* (2), and is probably caused by a different strain of the fungus (3). It is more severe in some years than in others (11). Soil fumigation provides partial control (1, 4), but little has been reported about host and environmental factors of the disease or its distribution in time and space. Better knowledge of these would probably lead to more effective control measures. This report deals with some factors of immediate interest to the nurseryman, including nursery conditions, seed, and seedling characteristics.

MATERIALS AND METHODS.–*Nursery observations.*–Standard seedbeds in nurseries are 1.1 m wide by 15.3 m long, laid out in parallel rows 0.46 m apart with long axes running east-west. Beds may be without shade or side protection (open), or surrounded by 20-cm-high side boards and shaded by lath spaced 5 cm apart (closed). Each seedlot (provenance) is sown in a separate bed. Sowing is done in spring (April-May) or fall (October-November) using, at the time of this investigation, a hand-broadcasting method designed to produce a uniform stand of 430 seedlings/m^2 (40/ft^2) but, in fact, resulting in densities one-half to twice this figure.

To study the effects of different seedlots and sowing methods on the incidence of root rot (percent seedlings killed), different seedlots were sown in adjacent beds. Some of those spring-sown in open beds were also sown in closed beds the following fall. Seedlings killed by root rot were counted monthly in 1.1 × 0.85 m (1 ft^2) plots placed randomly across seedbeds and divided into north and south subplots. The investigation was continued for 4 years, using up to 10 seedlots each year in each of two nurseries at Duncan and Green Timbers. The effect of seedling stand density on root rot incidence was studied by sowing varying numbers of seeds/m^2 (215, 430, 645, 860, 1,075, and 1,219) in 0.9 × 0.6 m plots. Spatial distribution of the disease was recorded by photographing plots from above to show diseased seedlings in relation to a superimposed 5-cm grid. Correlation of seedling growth with disease incidence was studied by weighing and measuring 18 seedlings from each plot immediately after the shoots had become dormant.

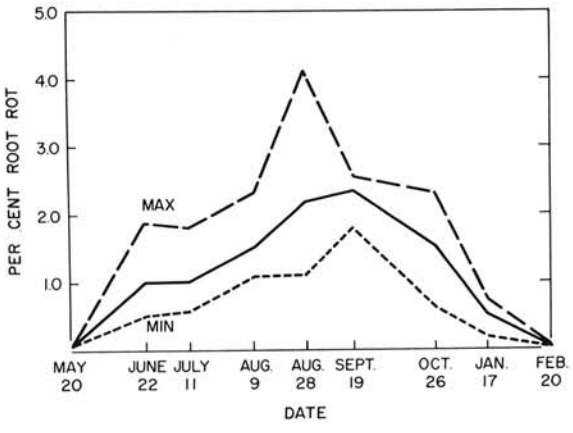

Fig. 1. Percent root rot disease at monthly intervals at Duncan nursery. Min and max denote percent in plots with least and most disease, respectively; mean is average of all plots.

Incidence of root rot varied among seedlots and years at both nurseries, ranging from 0 to 21%. Soil variation did not contribute significantly to seedlot differences, as indicated by lack of interactions between seedlots and seedbeds. Disease was consistently higher at Duncan (3-20%) than at Green Timbers (1-2%), and was generally less in the southern half of enclosed beds than in the northern half. It varied significantly among seedbeds, but not among plots within each bed. Disease in spring-sown open beds (6-9%) was higher than in fall-sown closed beds (0-4%). Density of seedling stand had no effect on disease incidence. The disease occurred from May to February, with maximum incidence in mid-August to mid-September (Fig. 1). Pielou's test (9) showed that diseased seedlings occurred randomly in the seedbeds. Pielou & Foster's test (10) showed that isolated seedlings were more liable to the disease than those in clumps.

Disease incidence was not correlated with the height above sea level at which the seed had been harvested or with seedlot germination in standard presowing tests. There were no significant correlations between disease incidence and mean shoot length, shoot weight, root length, or root weight per plot.

Greenhouse experiments.–Experiments were conducted as follows to investigate the effects and interactions of temperature regime and soil source on root rot caused by *F. oxysporum*. Six-inch pots containing Duncan or Green Timbers nursery topsoil were each sown with five seeds of the same lot, and placed in one of the following greenhouse or growth chamber environments: (i) temperature 20 ± 3 C for 4 months, 14 hr/day supplemental light (moderate regime); (ii) temperature 24 ± 0.5 C, 2,000 ft-c light for 12 hr, alternating with 18 ± 0.5 C in darkness for 12 hr (warm regime); (iii) temperature cycled diurnally in the 1st month from 3 to 25 C, in the 2nd month from 4.5-27 C, in the 3rd month from 7-32 C, and in the 4th month from 6-30 C, variation ± 0.5 C. Light increased to 2,000 ft-c diurnally for varying periods each month, simulating the mean Duncan nursery temperature and light conditions from May to September (nursery regime). In all regimes, light source was 76% cool-white fluorescent and 24% incandescent by wattage. Relative humidity ranged from 50 to 80%. Pots were watered to saturation daily.

Root rot was increased significantly (from 10 to 43%) only in Duncan nursery soil in the warm temperature regime. In all regimes, the number of pots containing one diseased seedling was significantly greater than those containing two or more ($P = .05$).

The effect of seedling age on incidence of root rot caused by *F. oxysporum* was investigated by

transplanting seedlings of various ages into artificially infested soil as follows. Polystyrene tubes, 9 cm long by 2 cm in diam (tree planting bullets), were first split longitudinally, and the halves were rejoined and held together by a plastic collar. The bullets were filled with nursery topsoil mixed with an equal volume of coarse sand to reduce compaction, and were then fumigated with methyl bromide (2). After ventilation, the tops of the bullets were capped and the bases wrapped with sterile aluminum foil. They were watered by immersion in sterile water. A Douglas-fir seed, surface-sterilized by immersion in 0.1% mercuric chloride aqueous solution and rinsed 3 times with sterile water, was sown in each bullet. The sowing was staggered to obtain seeds and seedlings of various ages by the same transplanting time; i.e., seeds 5 or 10 days after sowing, and seedlings 0, 5, 10, 15, or 20 days after emergence, or 10 or 30 days after formation of true needles. Temperature was cycled from 18.5 to 24.0 C diurnally, and light was cycled to 2,000 ft-c for 12 hr/day.

Seed or seedlings were transplanted into soil infested by *F. oxysporum* using the following method. Polystyrene cups, 8 cm in diam by 11 cm deep, were filled with soil-sand mix. A polystyrene planting bullet was pressed into the soil to form a transplanting hole. The cups were fumigated with methyl bromide, and after ventilation, each cup received 10 ml of a sterile water spore suspension of *F. oxysporum*, root rot strain, derived from a hyphal tip culture. Concentration of macrospores plus chlamydospores was 10,000/ml. Some cups received water only. Cups were covered with sterile petri lids and incubated 6 weeks in darkness in temperatures cycling diurnally from 18.5 to 24 C. Soil moisture was maintained gravimetrically at 60% of maximum moisture content, using sterile water. The establishment of *F. oxysporum* in the soil was verified by culturing soil particles on 2% malt extract agar.

Transplanting without damage to roots was effected by removing one side of a seedling bullet, then sliding the entire soil plug plus roots, supported on the other side, into the hole left by the removal of a same-sized bullet from the soil in the cups. Treatments were randomized in 22 blocks. Seedlings killed by root rot were recorded daily. After 5 months, seedlings were removed from the cups intact; root length and root lesion length were measured. Lesions were expressed as a percentage of the aggregate length of all roots longer than 5 mm. Tissue at the margin of lesions was surface-sterilized in an ultrasonic cleaner and planted on 2% malt extract agar for reisolation of fungi.

Incidence of root rot was highest in plants transplanted to infested soil as seed, and generally diminished as the age at transplanting increased (Table 1). Virtually no root rot occurred in seedlings transplanted 15 days or more after their emergence. The number of days after transplanting at which shoot symptoms of the disease appeared varied with each age class, but showed no trend. The proportion of the root system killed was similar for all age classes but one (10 days after seeding). *Fusarium oxysporum* was isolated from all lesions. Seedlings in uninfested soil had small numbers of lesions from which bacteria and saprophytic fungi were isolated. There was no correlation between shoot size and proportion of root system killed.

Soil population studies.—To investigate correlations between root rot incidence and soil microflora populations, 1:10,000 or 1:100,000 dilutions of soil suspension were prepared from thoroughly mixed soil in each pot from the foregoing temperature regime-soil source experiment. One ml of the suspension was incorporated in each of the

TABLE 1. Mortality and root lesions in Douglas-fir seedlings sown or transplanted at different ages into soil infested with *Fusarium oxysporum*

Age when transplanted into infested soil	No. days after seeding	% Killed[a] by root rot	Mean no. days after transplanting until killed	% Root length showing lesions
Seeded	0	41.0	75	40.1
5 days after seeding	5	27.2	58	31.8
10 days after seeding	10	25.0	87	19.2[b]
At emergence	20	15.0	112	30.5
5 days after emergence	25	5.0	58	30.5
10 days after emergence	30	13.0	79	29.8
15 days after emergence	35	0.0		29.8
20 days after emergence	40	0.0		34.8
10 days after true needles formed	50	0.0		29.8
30 days after true needles formed	70	4.3	107	27.9
Uninfested soil				
Seeded	0	0.0		9.8[b,c]
20 days after emergence	40	0.0		6.4[b,c]

[a] Values greater than 15.0% were significantly greater than smaller values ($P = .05$)
[b] Significantly less ($P = .05$) than other lesion percent values.
[c] Bacteria and saprophytic fungi isolated from lesions.

TABLE 2. *Streptomyces* and fungus populations in different nursery soils and temperature regimes

Regime	Soil	Colonies/g oven-dry soil (X 10^4)			
		Phycomycetes	*Penicillia*	Total fungi	*Streptomyces*
24 hr at 20 ± 3 C for 4 months	Duncan	1	4	8	3
	Green Timbers	0	2	4	17
24 hr at 20 ± 3 C for 4 months	Duncan	11	8	30	11
	Green Timbers	1	4	26	13
12 hr at 24 ± 0.5 C, 12 hr at 18 ± 0.5 C for 4 months	Duncan	3	4	22	13
	Green Timbers	0	1	14	63

following media: 2% malt extract agar, soil extract agar, or peptone-rose bengal-streptomycin agar. Fungus colonies were counted and identified to genus after incubation for 5 days at 20-23 C. Actinomycetes were counted and identified by Waksman's morphological classification (13) after 3 weeks' incubation.

Fungus populations, especially phycomycetes and penicillia, were consistently higher in Duncan soil than in Green Timbers soil, whereas *Streptomyces* populations were consistently higher in Green Timbers soil (Table 2). *Fusarium oxysporum* populations did not differ between nurseries. A remarkable increase in *Streptomyces* occurred in Green Timbers soil in the warm temperature regime. No marked differences occurred between soils in the proportions of *Streptomyces* groups. The most frequent were Waksman's groups 2, 3, and 5.

DISCUSSION.—Root rot disease is partly affected by host genotype, since different seedlots represented genetically distinct populations, but it seems to be independent of population vigor factors such as germinability and seedling growth. Seedling age is an important factor in root rot incidence, as indicated in previous experiments (2). Greatest disease occurred in seedlings exposed to infection from the time they were sown, even though symptoms did not appear until at least 2 months later. Since a similar proportion of root system was killed in all age classes, incidence of root rot disease must depend on seedling tolerance to loss of roots, the youngest seedlings being the least tolerant. The similarity of root proportion killed implies that there are, regardless of seedling age (i) the same proportion of infection sites; (ii) equal probability of infection; and (iii) the same lesion growth rate.

Root rot incidence was greatly increased by the warm regime which was characterized by a temperature of 24 C or higher for more than 6 hr/day during the 1st month after sowing. This effect undoubtedly results from the maximum activity of *F. oxysporum* at 25 C (12) during the seedling stage when root rot susceptibility is highest. Lower temperatures during this period, or higher temperatures after it, had no effect. In 1963, the worst year for the disease, Duncan nursery had the highest May temperature and the most hours of bright sunshine of the 4 years in which samples were taken (5). Measures which reduce soil temperature below 24 C also reduce disease incidence; e.g., fall sowing, which results in early spring germination when temperature is below 21 C, or shading by overhead laths or sideboards on the south edge of beds.

The effect of temperature on root rot incidence was modified in Green Timbers soil, which contained a high population of *Streptomyces* spp., well-known antagonists of *Fusarium oxysporum* (7, 8). Furthermore, Green Timbers nursery received an average of 30 hr less bright sunshine in May than did Duncan (5). Both factors probably contribute to the control of disease in this nursery.

The increase in disease incidence from May to August and its subsequent decline to February are probably due to the variation in seedling germination rate which, in turn, affects the period in which infection can occur. Thus, the frequency distribution for germination and disease incidence are similar, although the latter extends over a longer time due to delay between root infection and symptom expression. Also, postinfection temperatures probably affect the rate of lesion growth, accelerating the appearance of symptoms in early summer and delaying it in the fall.

The random spatial distribution of root rot disease probably results from thorough mixing of inoculum in the soil during preparation of seedbeds by rototiller. Soil preparation proceeds lengthwise in each bed, tending to distribute inoculum evenly within a bed. The difference in disease incidence among beds is probably due to local accumulations of inoculum in piles of rejected seedlings and residues of pruned roots. The initial random infections are not followed by spread of the disease from one seedling to another. This was implied by the preponderance of pots with one diseased seedling compared to pots with two or more diseased seedlings. The greater disease susceptibility of isolated seedlings may be due to their root systems occupying a larger soil volume than those in groups, increasing the probability of inoculum contact.

Practical implications.—Practical applications of these results in reducing Douglas-fir root rot disease in nurseries include: identification of those seedlots that are less resistant to the disease than others so

that they can be given special protection, or can be sown in low-risk areas; keeping temperature below 24 C during the 1st month after sowing by shading, irrigation, etc.; concentration of phytoprotection measures, e.g., fungicidal soil drenches during this critical period for infection; and elimination of inoculum sources by sanitation.

Further investigation should be undertaken into the role of *Streptomyces* in root rot disease.

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