# Distribution and Pathogenicity of Fusarium oxysporum in a Forest Nursery Soil

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ABSTRACT

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Diseased roots of Douglas-fir seedlings killed by Fusarium root rot in a nursery the previous growing season were cultured to determine the presence of *Fusarium oxysporum*, and the pathogenicity of the Fusarium isolates was determined on Douglas-fir seedlings. All root segments yielded the fungus and about 80% of the isolates were pathogenic. Particulate organic matter, consisting mainly of root fragments, sawdust, and unidentified particles, was collected by flotation from soil cores taken from the nursery. Most root fragments were < 2 mm long and the unidentified particles were < 1 mm long. There was no significant difference in the distribution of any of the particle types by depth in soil. Incidence of *F. oxysporum* in all particle types was variable, but significantly lower than in diseased roots. The incidence of *Fusarium* was significantly lower in root fragments that were shorter than 4 mm. Sawdust had the lowest incidence of *F. oxysporum*. Pathogenicity of *Fusarium* spp. isolates from the unidentified particles was significantly lower than those from roots. Temperature had no significant effect on pathogenicity of *F. oxysporum* from diseased roots over the range 10-30 C.

*Additional key words: Pseudotsuga menziesii.*

Parkinson and Thomas (9) found that propagules of *Fusarium* spp. in soil were largely confined to soil organic matter. Nash et al. (8) showed that chlamydospores of *F. solani* f. sp. *phaseoli* originated within the infected bean root and concluded that these were the main source of inoculum in the soil. Pathogenicity of *Fusarium* appears to depend on contact or near-contact with host roots (6). Thus, probability of host infection may be affected by pathogenicity and number of pathogen propagules in the soil. Inoculum density is related to the size and distribution of colonized particles in the soil (2, 3). In studying root rot of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] nursery seedlings which is caused by *Fusarium oxysporum* Schlecht., my objective was to ascertain how inoculum factors were related to disease epidemiology.

## MATERIALS AND METHODS

This study was carried out in a forest nursery at Duncan, B.C., that had been continuously cropped with Douglas-fir seedlings for 10 years.

**Inoculum estimation.**—Two types of inocula were tested for the presence and infectivity of *F. oxysporum*. (i) Diseased roots of 1-year-old seedlings killed by root rot were collected for two consecutive years from random locations in several nursery beds in the spring (May), following the growing season in which they were killed. Roots covered with black lesions typical of the disease were washed in tap water for 5 minutes, gently scrubbed with a camel's-hair brush to remove soil particles, and cut into 5-mm lengths. (ii) Particulate organic matter was collected from soil cores (2 × 20 cm) taken from a representative 15-m-long bed that had been prepared and treated for growing seedlings, but had been left unsown. Cores were collected at seed-sowing (May 1973), after the end of the first growing season (December 1973), and in May and July of the following year. On each date, five cores spaced equally across the 1.1-m-wide bed at a randomly chosen location were removed with a soil corer. A slow, even pressure was applied to avoid lengthwise compression of the core. Each core was placed in a polyethylene pipe and plugged at both ends to minimize moisture loss and microbial contamination.

In the laboratory, each core was subdivided into ten 2-cm-long segments. Each segment was processed as follows: lumps were crushed, pebbles removed, and the soil was stirred into 100 ml of a mixture of glycerol and water (3:1, v/v). Over 90% of the particulate organic matter in this nursery soil floated off in this mixture. After a 24-hour settling period, the floating material was siphoned off and collected on filter paper, where it was washed twice with 10 ml of sterile distilled water. The paper and contents were air-dried and placed in a closed dish at 2 C.

Extracted material from each 2-cm segment was classified by microscopic examination as: (i) root fragments, 0.5-3 mm in diameter, of unidentified species but clearly possessing root structure; (ii) sawdust, undoubtedly from annual mulching of beds; and (iii) unidentified particles usually less than 1 mm. These three types accounted for over 95% of the material collected. Weed seeds, conifer needle fragments, and cone scales accounted for the balance. To gain some idea of size class distribution, all unidentified root fragments in the May 1973 and May 1974 samples were measured and classified by 2-mm length classes up to 12 mm and a 12+ mm class.

**Fungus incidence.**—Incidence of *F. oxysporum* in each type of organic particle was determined by plating all

those from each core on soil extract agar (7) adjusted to pH 5.5. This medium was superior to several *Fusarium*-selective media for inducing macrospore formation. Isolates were identified by comparison with cultures of *F. oxysporum* identified by C. Booth, Commonwealth Mycological Institute. Incidence of the fungus in diseased Douglas-fir roots was tested in both years of collection by plating, respectively, 130 and 100 randomly selected 5-mm segments from about 40 seedlings.

Incidence of *F. oxysporum* in soil residues after extraction of the particulate organic matter was determined in the May and July 1974 collections by pouring the glycerol-water mixture off the settled soil residue from each 2-cm segment and serially diluting the residue with sterile distilled water to a final dilution of 1/10,000. Triplicate samples were drawn for each segment and duplicate subsamples were incorporated by swirling into cooled molten soil-extract agar. Colonies that developed at 23 C were examined for presence of *F. oxysporum*. Previous tests of this soil without the extraction procedure showed that a 1/10,000 dilution produced up to 10 colonies of the fungus per plate and that keeping the soil in suspension for 24 hours did not affect the number of *F. oxysporum* colonies.

**Pathogenicity**.—Pathogenicity was tested within the temperature range usually encountered in the nursery (10-30 C), using diseased root segments, unidentified root fragments and particles, and clumps of 5-10 macrospores with some adhering agar as inoculum. Insufficient *F. oxysporum* for testing was obtained from sawdust or soil residue. Tests with diseased roots were conducted on randomly chosen segments collected in both years, using constant temperatures 10, 15, 25, and 30 C.

Because unidentified root fragments had such a low incidence of *F. oxysporum*, the tests used material representing a range of soil depths and sizes, selected after the fungus grew from them on soil-extract agar. The particle was then rinsed with sterile distilled water before use.

Unidentified particles were tested with day/night temperatures of 25/20 C. Inoculum was taken from a range of soil depths and the particles were selected after *F. oxysporum* was found to be present.

Light conditions for all tests were 12 hours light supplied by cool-white fluorescent lamps at 21,520 lx.

Inoculations were made by placing inoculum in contact with radicle tips of 10- to 20-day-old seedlings grown axenically in perspex observation boxes containing translucent acrylic beads (4). Inoculations were made through a port inside of the box after carefully removing a few beads to expose the radicle. Inoculated seedlings were examined every 5 days and the following responses were noted: lesion formation as denoted by a brown spot on the fleshy white root, accompanied by collapse of tissues, and damping-off denoted by collapse of the seedling shoot. Thirty days after inoculation, the inoculum and portions of each seedling were excised from the inoculation region, surface sterilized in aqueous solution of 0.5% available chlorine for 5 minutes, rinsed with water, and plated on Difco 2% potato-dextrose agar to determine the presence of *F. oxysporum*. Each test included uninoculated controls and each treatment was

TABLE 1. Frequency of organic particle types and incidence of *Fusarium oxysporum* in a nursery soil

| Particle types and collection dates | Particles per core[a] (no.) | Particles yielding *F. oxysporum*[a] (%) |
|---|---|---|
| Unidentified root fragments | | |
| May 1973 | 12.3 x | Not tested |
| December 1973 | 7.1 y | 0 w |
| May 1974 | 4.3 y | 22.6 x |
| July 1974 | 7.7 y | 8.5 y |
| Unidentified particles | | |
| December 1973 | 11.6 x | 0 w |
| May 1974 | 11.2 x | 5.3 y |
| July 1974 | 8.1 xy | 13.0 z |
| Sawdust | | |
| May 1974 | 37.9 z | 0.5 w |
| July 1974 | 38.1 z | 4.0 y |

[a]Values are means of five cores (20 × 2 cm). They differ significantly ($P = 0.05$) if followed by different letters. Means were compared by Duncan's multiple range test for particle numbers and by the chi-square test for incidence of *F. oxysporum* in the particles.

TABLE 2. Pathogenicity of different types of *Fusarium oxysporum* inocula from a forest nursery soil

| Inoculum type and collection dates | Temperature (C) | Pathogenicity (%)[a] | |
|---|---|---|---|
| | | Particle[b] | Macrospores[c] |
| Diseased root | | | |
| May 1973 | 25 | 92 y | Not tested |
| May 1973 | 30 | 79 xy | Not tested |
| May 1974 | 10 | 85 y | 100 z |
| May 1974 | 15 | 86 y | 92 yz |
| Unidentified root fragments | | | |
| May 1973 | 30 | 77 x | Not tested |
| May 1974 | 25/20 | 95 y | 75 x |
| Unidentified particles | | | |
| May 1974 | 25/20 | 31 w | 77 x |

[a]Values are significantly different ($P = 0.05$) by the chi-square test if followed by a different letter.
[b]Particles used as inoculum.
[c]Macrospores derived from particles used as inoculum.

replicated with 10-15 seedlings.

## RESULTS

The frequency of each particle type was expressed as the average number per soil core (Table 1). Sawdust particles were most numerous and unidentified root fragments were generally least numerous.

Incidence of *F. oxysporum* was expressed as the percentage of particles yielding the fungus (Table 1). The incidence varied significantly ($P = 0.05$) among collection dates and particle types, but unidentified root fragments tended to have the highest and sawdust the lowest incidence.

There were no significant differences in the distribution of particle types at various soil depths. Nearly 80% of the unidentified root fragments were less than 4 mm long but incidence of *F. oxysporum* was significantly lower in them than in fragments over 4 mm long (17 vs. 39%, respectively). Incidence of the fungus was 100% in diseased roots and 0% in soil from which the particulate organic matter had been removed.

Disease production by *F. oxysporum* from unidentified particles was significantly lower than that from diseased roots, and unidentified root fragments (Table 2). Macrospore inoculum from diseased roots was significantly more effective in disease production than spores from other sources. Different temperature regimes (Table 2) for the different inocula limit comparisons that can be made.

## DISCUSSION

Diseased roots of seedlings killed by Fusarium root rot in previous crops are highly suspect as the major inoculum source for the disease because incidence and pathogenicity of *F. oxysporum* were high in them. Because seedling roots comprise the great majority of root material in nursery soil, it is likely that unidentified root fragments come from this source, probably breaking down to shorter lengths with yearly rototilling of the beds. This operation also would tend to distribute the fragments uniformly by depth. The relatively low incidence of *F. oxysporum* in root fragments, especially the shorter ones, is compatible with the concept of reduced propagule survival in decomposed host tissue (6). The variation in incidence of *F. oxysporum* in these fragments at different collection dates probably reflects the patchy distribution of the disease (5) rather than seasonal effects.

Probably part of the unidentified particles also are derived from seedling roots since, other than conifer sawdust, little organic matter has been applied to these beds. The somewhat lower incidence and much lower pathogenicity of *F. oxysporum* in this type of inoculum further exemplifies the effect of host tissue decomposition on infectivity. Apparently, there is little change in the concentration of any particle type during a 2-year rotation. The single exception (root fragments, May 1973) was probably attributable to a local concentration of material, rather than a seasonal effect.

In practice, when removal of diseased seedlings is economically feasible; e.g., during hand weeding, it should be done. Otherwise decomposition of diseased roots should be encouraged by thorough cultivation of diseased spots during fallow periods. Soil from diseased spots should not be spread to other areas. Fumigation also will have a greater chance of success if diseased roots are fragmented before treatment (1). Use of sawdust mulch, advocated by Wright et al. (10), for control of root rot appears to confirm the low importance of this material as an inoculum source.

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