Strain Differences in Fusarium oxysporum Causing Diseases of Douglas-Fir Seedlings

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ABSTRACT

Axenically grown Douglas-fir seedlings were inoculated with *Fusarium oxysporum* isolates obtained in a nursery from roots of healthy Douglas-fir seedlings or from roots of seedlings infected with pre-emergence damping-off, postemergence damping-off, root rot, or corky root. All isolates killed all seedlings, but there were significant differences among isolates in the chronological pattern of mortality they produced. In seedlings growing in controlled environments in naturally infested soil, there was no significant association between any one disease caused by *F. oxysporum* and another. In nurseries, there were no significant correlations between the levels of one disease and another.

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Evidence from artificial inoculation experiments and from field observations (1) suggested that although pre-emergence damping-off, postemergence damping-off, and root rot of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings in nurseries were all caused by *Fusarium oxysporum* Schlecht., nevertheless the diseases seemed to occur independently, and fungus isolates from seedlings with different symptoms were rather specific in the type of symptom they caused in inoculation tests. Furthermore, isolates from healthy nursery seedlings proved pathogenic in inoculation tests.

Differential pathogenicity of *F. oxysporum* to conifer seedlings has been reported previously. Vaartaja & Cram (10), isolating *F. oxysporum* var. *redolens* from both damped-off and healthy individuals, concluded that some *Fusaria* may be weakly pathogenic. Matuo & Chiba (4) reported

differences in pathogenicity among isolates of *F. oxysporum* f. sp. *pini*, whereas Tint (9) differentiated pathogenicity of several *Fusaria* included in *F. oxysporum* sensu Snyd. & Hans. On the other hand, Shea & Rediske (6) believed damping-off and root rot of Douglas fir were caused by the same *Fusarium*.

Although strain variation in *Fusarium oxysporum* is possibly an important consideration in disease control, its detection is beset by two difficulties: under natural conditions, pathogenic differences due to strain may be confounded with other variations; e.g., inoculum concentration, seedling resistance, or environmental conditions, whereas artificially controlled conditions may limit the range of application of results. Therefore, three complementary methods were used to detect strain differences: (i) Pathogenicity of representative isolates of each disease was tested by inoculating seedlings under closely controlled conditions; (ii) the association of one disease with others in controlled environments in naturally infested soil was determined; and (iii) correlations between different diseases in nurseries were statistically analyzed.

Artificial inoculations.—Douglas-fir seedlings were grown axenically on nutrient agar in culture tubes (1) from seed of uniform size and weight. Tubes, each containing one seedling, were placed in a growth chamber with a diurnal cycle of temperature and light (Table 1).

Ten samples of each of the following types of Douglas-fir material were collected randomly from a nursery: (i) seeds that failed to germinate after sowing and showed symptoms of pre-emergence damping-off; (ii) fresh postemergence damped-off seedlings; (iii) roots of root-rotted 1-year-old seedlings (root rot); (iv) roots of root-rotted seedlings that remained in the nursery bed 1 year after death (residual root rot); (v) roots of 2-year-old seedlings with "corky-root" (2); (vi) roots of healthy seedlings about 8 weeks old after damping-off had ceased (survivors of damping-off); and (vii) roots of healthy 2-year-old seedlings (root rot survivors).

All samples except those showing corky root were collected from the same provenance as seedlings in culture tubes.

Isolates of *Fusarium oxysporum* from each sample were grown on 2% malt extract agar for 5 days; then hyphal tips were transferred to soil extract medium containing 1% agar, pH 5.5 (the same pH as in the culture tubes) and incubated until macrospores and chlamydospores were well developed. The cultures on 9-cm-diam agar discs were homogenized each with 25 ml sterile distilled water in a blender at slow speed for 3 min, and the suspension was allowed to stand for 3 days. All culturing was done in darkness at 25 C. On the basis of nine samples from each suspension, concentration of spores (chlamydospores plus macrospores) was diluted to 20,000/ml, then 0.1 ml was pipetted onto the agar surface in each culture tube containing a seedling. The procedure, in duplicate, was carried out at the same time of day (i) 7 days after planting the germinating seed (cotyledonary seedlings); or (ii) 7 days after full development of the first true needles (postcotyledonary seedlings), at ca. 8 weeks. By daily microscopic examination, date of seedling death by destruction on the hypocotyl, invariably sudden and complete, could be precisely fixed.

TABLE 1. Temperature and light conditions during inoculation of Douglas-fir seedlings with various *Fusarium oxysporum* isolates

Time (hr)	Ambient temp (C)	Time (hr)	Light (ft-c)
0400-1200	Constant rise from 13 to 26	0400-0520	500
		0520-0640	1,000
		0640-0800	1,500
1200-1600	26	0800-1600	2,000
1600-2400	Constant fall from 26 to 13	1700-1820	1,500
		1820-1940	1,000
		1940-2100	500
2400-0400	13	2100-0400	0

All inoculated seedlings were killed, and no noninoculated seedlings died. Data on length of survival period were statistically analyzed. Transformation appropriate to the type of distribution caused only trivial changes in statistical significances; therefore, original means are shown in the tables, tested for significant differences by Duncan's multiple range test. Differences were highly significant, seedlings surviving twice as long in some treatments as in others (Table 2). There were some highly significant interactions between source of inoculum and time of inoculation: in two treatments, cotyledonary seedlings survived longer than postcotyledonary ones; in another treatment, the postcotyledonary seedlings survived longer, whereas in four treatments there were no differences. However, these interactions did not obscure the main differences among inoculum sources, survival being longest in residual root rot and longer in postemergence damping-off than in the other treatments. There were no significant differences within the 10 isolates from each type of disease or between replications of each isolate.

Isolates from different disease types were also compared with respect to time-spread of mortality between the first and last seedling death, estimated by the variance, or scatter of survival days about the mean. Using Scheffé's test for heterogeneity of variances of non-normal distributions (7), there was significantly greater scatter in survival days of cotyledonary seedlings inoculated with residual root rot or survivors of damping-off inocula, and significantly less scatter in postcotyledonary seedlings inoculated with corky root inoculum when compared to the remaining treatments.

The chronological patterns of mortality caused by isolates from different disease types were compared using tests for skewness (8) and departure from normality (5) of the frequency distributions of mortality upon time. Distributions associated with corky root and surviviors of damping-off inocula featured significant skew caused by high mortality

TABLE 2. Mean days Douglas-fir seedlings survived, and variance of survival days after inoculation with *Fusarium oxysporum* isolates from different sources

Source of isolates	Mean days survived after inoculation		Variance of days survived after inoculation	
	Cotyledonary seedlings	Postcotyledonary seedlings	Cotyledonary seedlings	Postcotyledonary seedlings
Diseased seedlings				
Pre-emergence damping-off	8.2 ad[a]	7.7 d	3.22 cd[a]	2.74 cd
Postemergence damping-off	11.5 b	10.3 b	4.03 cd	3.41 cd
Root rot	9.0 a	8.9 ab	2.58 cd	2.67 cd
Residual root rot	15.5 c	11.0 e	10.09 ab	4.47 bc
Corky root	7.8 ad	8.1 ad	4.52 cd	1.70 de
Healthy seedlings				
Survivors of damping-off	9.4 a	7.4 d	13.03 a	2.61 cd
Survivors of root rot	9.4 a	11.8 e	2.03 cd	2.76 cd

[a]Significant differences ($P = .05$) occurred if no letters following are common to the values being compared.

soon after inoculation, tapering off with time. Others were fairly symmetrical in that mortality increased regularly to a peak, then declined regularly (Fig. 1).

Speed with which seedlings were killed by isolates from different disease types was estimated by the linear regression coefficients, or slopes, of probit of percentage killed on the logarithm of the number of days after inoculation. Comparison of coefficients by Scheffé's S-method for multiple comparisons (A. W. Douglas, *personal communication*) indicated significantly greater slope; i.e., faster killing by isolates from survivors of root rot (b = 14.83) and significantly slower killing by isolates from pre-emergence damping-off (b = 7.84) when compared to isolates from other disease types.

Natural infection.—Interdependence of pre-emergence damping-off, postemergence damping-off, and root rot was investigated in Douglas-fir seedlings in pots containing soil from two nurseries, 40 pots of each soil, five seeds sown/pot. The experiment was repeated in three environments; a greenhouse in which temperature ranged from 18 to 22 C; a growth chamber with a constant day:night temperature of 23.5:18.5 C; and a growth chamber in which temperature changed diurnally and also monthly to simulate conditions in the nursery during the summer; viz, 1st month, 3 to 24 C; 2nd month, 4.5 to 27 C; 3rd month, 6.5 to 32 C; 4th month, 6 to 30 C. Greenhouse experiments received 14-hr supplemental fluorescent light; light in growth chambers was programmed to change diurnally from darkness to 2,000 ft-c. Postemergence damping-off and root rot disease were recorded daily; then, ungerminated seeds were recovered from the soil and dissected microscopically to determine the number killed by pre-emergence damping-off. Isolations were made from diseased seeds and seedlings. Association of one disease with another was tested by applying chi-squared tests of independence to the number of pots containing each type of disease.

In all environments and soils, the number of pots that either contained or were devoid of any two types of disease was not greater than would occur by chance ($P = .05$). *Fusarium oxysporum* was isolated from over 90% of the diseased seedlings.

Correlations between diseases in nurseries. — Relationships among diseases caused by *F. oxysporum* were investigated by correlation analysis of disease counts in nursery beds with seedling stand densities ranging from 140 to 810/m^2 (3) and in beds in another nursery in a different year with different seed provenances and soils (2). No significant correlations occurred between levels of one disease with that of another, expressed as either per cent of seedling stand or per cent germination, regardless of density of seedling stand. Moreover, there were no correlations between combined frequencies of two diseases with that of a third.

DISCUSSION.—The null hypothesis that no difference in pathogenicity occurred among isolates from each type of disease was rejected. The criteria, mean number, and scatter of survival days, chronological pattern of mortality, and differential response between seedling ages, were meaningful parameters of pathogenicity. The small variation among 10 isolates within each disease type randomly taken at two different times indicated a high probability of uniformity within disease types in the natural population. Lack of significance between replicates implied uniformity within each isolate. The experimental conditions, e.g., unnatural rooting medium for seedlings, heavy inoculum dose, ideal conditions for infection, probably reduced pathogenic differences among strains of *F. oxysporum* and accounted for lack of survivors. Under more natural conditions, isolates from different diseases differed significantly in the number of seedlings they killed (1). The effect of seedling age also differed in the two experimental conditions.

In pots of nursery soil containing natural *F. oxysporum* inoculum, two diseases caused by the same fungus strain should have been manifested as a significant number of pots that either contained or were devoid of both diseases. No two diseases showed a significant association, implying that they were caused by different strains. In nursery plots,

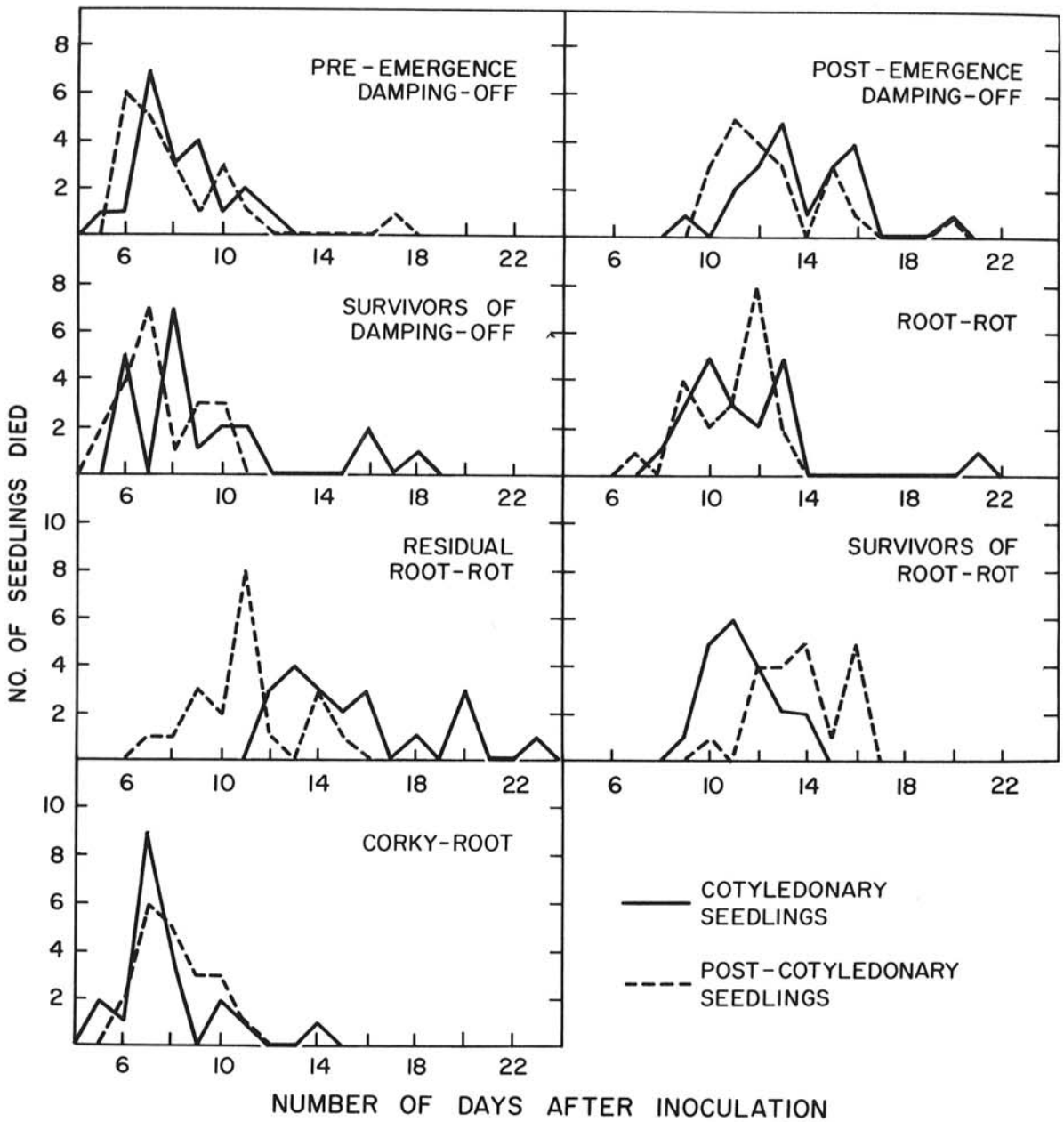

Fig. 1. Distributions of daily mortality in Douglas-fir seedlings of two ages after inoculation with *Fusarium oxysporum* isolates from various sources.

commonality of fungus strain to two or more diseases should have been manifested as a significant correlation of frequency of one disease with that of another, or with the combined frequency of two diseases. Therefore, since no correlations were found among levels of different diseases under a variety of nursery conditions, including very low to very high seedling populations, it must be assumed that the diseases were independent of one another and were caused by different strains of *F. oxysporum*.

The results of the several tests, therefore, mutually support the hypothesis for strain differences in *F. oxysporum*, and are also consistent in this respect with those obtained with artificial inoculations of *F. oxysporum* in soil (1).

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