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Research note

Fusarium root infection of container-grown Douglas-fir: effect on survival and growth of outplanted seedlings and persistence of the pathogen

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Application. Results of this study indicate Douglas-fir seedlings with low levels of *Fusarium* infection in their roots will grow and survive as well as non-infected seedlings after outplanting on a forest site.

Abstract. Greenhouse-grown Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seedlings with roots infected with either *Fusarium oxysporum* Schlect. or *Fusarium proliferatum* (Matsushima) Nirenberg were outplanted on a forest site in northern Idaho, U.S.A. No resident *Fusarium* populations were detected in the forest soil. *Fusarium* persisted the first four years on roots initiated during the greenhouse phase, but occurred sparingly or was absent on roots that grew after outplanting. Height growth was unaffected, and mortality was not often associated with *Fusarium*. A seasonal pattern of *Fusarium* activity was observed. Low levels (10–40%) of initial root infection apparently have little adverse effect on outplanting performance of Douglas-fir seedlings.

Introduction

Fusarium spp. are major pathogens of container-grown seedlings, especially Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco). These fungi can cause disease of container-grown seedlings throughout the growing season, especially eliciting pre- and post-emergence damping-off and root disease during bud initiation. Of the fusaria associated with container-grown Douglas-fir, *Fusarium oxysporum* Schlect. has generally

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been considered the most virulent (James et al. 1989). However, recent work indicates *Fusarium proliferatum* (Matsushima) Nirenberg may exhibit similar virulence and be more prevalent in container nurseries than *F. oxysporum* (James, unpubl. data).

Recent observations on Douglas-fir (James et al. 1987; James et al. 1988) determined seedling root infection by *Fusarium* is often not expressed as disease. The fate of infected, container-grown Douglas-fir seedlings lacking above-ground disease symptoms, and the *Fusarium* infections themselves, after outplanting is unknown. However, Smith (1967) found levels of *Fusarium oxysporum* on roots of outplanted, bareroot sugar pine (*Pinus lambertiana* Dougl.) seedlings decreased over time and after four years could not be detected.

Our study objectives were to evaluate the persistence of *Fusarium* on outplanted container-grown Douglas-fir roots and the role of *Fusarium* in causing seedling mortality after outplanting, and to determine resident populations of *Fusarium* in soil at the outplanting site.

Materials and methods

Douglas-fir seedlings were grown at the University of Idaho Forest Research Nursery in Moscow, Idaho (46.5 °N latitude and 117 °W longitude). This seedlot (IDL-T) was part of an earlier *Fusarium* study (James et al. 1987). During the earlier study, seedborne *Fusarium* and seedling infection levels were determined throughout the growing season. At outplanting, assays (described below) indicated 60% of the seedlings had about 10% of their root systems colonized by *Fusarium*, despite a lack of disease symptoms.

The Douglas-fir seedlings were operationally outplanted in April 1987 on a portion of the Flewsie Creek drainage near Clarkia, about 65 km northeast of Moscow. This area was harvested in spring 1986 and broadcast burned in fall 1986. Within the operational plantation, 187 seedlings were planted at 1.3-m by 1.3-m spacing. The site has a south exposure, 35% slope, is 975 m elevation, and the habitat type is classified as *Tsuga heterophylla/Clintonia uniflora/Clintonia uniflora* phase (Cooper et al. 1991).

Thirty systematically located samples of surface soil (0—10 cm) were collected at the time of planting. Soil was assayed for *Fusarium* using the dilution plate method (Nash and Snyder 1962), except Komada's (1975) selective medium was used.

Beginning in 1987, we sampled twenty seedlings each May and October through 1989. In 1990, we sampled 10 seedlings in May and again in

October. We considered root plugs as roots that grew during the greenhouse phase and maintained the shape of the container, while egressed roots were defined as all roots that grew away from the root plug. Roots from two locations on each seedling were sampled: 20 root pieces (3—4 mm long) from the root plug and 10 from egressed roots were randomly selected. Root pieces were washed, surface sterilized in a 1:10 bleach: water solution for 1 min, rinsed in sterile water, and aseptically placed on a selective medium for *Fusarium* (Komada 1975). These were incubated under cool fluorescent light at 22 to 24 °C for 7 days. Samples were assayed for infection, and percentage colonization of infected seedlings was calculated by counting root pieces colonized. Dead seedlings were also collected and assayed for *Fusarium* as described above, as well as evaluated for other possible mortality causes. Height of each sampled seedling was measured from groundline to the tip of the terminal shoot. Heights of infected and non-infected seedlings were analyzed with a one-way analysis of variance (Snedecor and Cochran 1980) and the means compared with Tukey's HSD.

Results and discussion

Persistence of Fusarium

Fusarium persisted on roots of Douglas-fir seedlings into the fourth year after outplanting (Table 1), but was not found by the end of the fourth season. Smith (1967) also could not recover *Fusarium* from bareroot sugar pine by the end of the fourth growing season. At lifting, 60% of the seedlings had about 10% of their root systems colonized (James et al. 1987). Our first sample, taken one month following planting, indicated 40% of the seedlings had nearly 44% of their root systems colonized. Although the total percentage of seedlings infected remained fairly constant for the first seven samples, the percentage of plug roots colonized by fusaria decreased over time and eventually became zero (Table 1). *Fusarium oxysporum* and *F. proliferatum* were the species most commonly found in root plug assays.

Fusarium was uncommon in egressed roots, and was found only in assays on four sample dates. Of these, *F. oxysporum* or *F. proliferatum* were recovered on only two of these four sample dates. Infection by *Fusarium* following outplanting probably did not occur, since assays of soil at the study site failed to reveal the presence of these fungi. When fusaria were assayed on egressed roots, it is likely the fungi colonized short distances from the plug roots. However, the fusaria did not persist on egressed roots, probably because of competition from other microflora.

Table 1. Persistence of *Fusarium* spp. on Douglas-fir seedling roots and heights of seedlings sampled after outplanting. All table values are means based on 20 seedlings for each sample from 1987 through 1989 and 10 seedlings for the two samples in 1990

		Plug roots colonized[2]		Egressed roots colonized[3]		Seedling heights		
Sample date	Seedlings infected[1] (%)	Total (%)	With *F. oxysporum* or *F. proliferatum* (% of total)	Total (%)	With *F. oxysporum* or *F. proliferatum* (% of total)	Infected seedlings (cm)	Non-infected seedlings (cm)	Average for all seedlings (cm)
1987								
May	40	43.8	74.3	0	0	18.4	16.6 NS[4]	17.3
October	50	27.0	77.8	0	0	25.3	27.6 NS	26.5
1988								
May	35	24.3	70.6	10	28.6	29.6	26.8 NS	27.8
October	20	15.0	66.7	0	0	38.7	41.6 NS	41.0
1989								
May	40	26.2	85.7	7.5	50.0	52.1	51.4 NS	51.7
October	30	13.3	100.0	1.7	0	65.8	67.2 NS	66.8
1990								
May	40	2.5	100.0	7.5	0	67.8	50.0 S	57.1
October	0	0	0	0	0	NA[5]	90.3	90.3

[1] Percentage of seedlings sampled with *Fusarium* spp. colonizing their roots.
[2] Average percentage colonization of roots from the original seedling plug with *Fusarium* spp. (infected seedlings only).
[3] Average percentage colonization of new roots, egressed from the original seedling plug, with *Fusarium* spp. (infected seedlings only).
[4] Average heights for infected and non-infected seedlings at each sample date followed by NS are not significant at the $p \leqslant 0.1$ level, but the May 1990 sample was significant (S) at the $p \leqslant 0.05$ level. The large increase in height between October 1988 and May 1989 is attributable to the spring flush of growth that occurred prior to the May 1989 sample.
[5] Not applicable since all seedlings were non-infected.

A seasonal pattern of plug root colonization was apparent in the greenhouse, and this pattern persisted after outplanting for four growing seasons, with higher colonization rates detected in May than in October. This pattern is similar to that detected for Douglas-fir ectomycorrhizae in the humus and mineral soil layers on forested sites (Harvey et al. 1978). Our assays for *Fusarium* failed to reveal whether the fungus was actively growing in roots or in a dormant state. It is possible spring samples detected mostly dormant infections, i.e., *Fusarium* in the form of chlamydospores or sclerotia within root cortical cells. As the growing season progressed and environmental conditions became more conducive to *Fusarium* development, these dormant infections probably became active. During active growth, *Fusarium* spp. are more susceptible to competition with, and antagonism by, other soil microorganisms (Baker and Cook 1974). By the end of the growing season, *Fusarium* infections may have been substantially reduced because of competitor/antagonist action. Levels of activity by some important competitors, such as *Trichoderma* spp., are greatly enhanced during the summer and reduced during the winter (Papavizas 1985). This may give *Fusarium* a slight competitive advantage during winter, resulting in consistently higher levels of detection in the spring.

Height and mortality of outplanted seedlings

Average height of infected and non-infected seedlings was similar after outplanting (Table 1). For all but the May 1990 sample, a significant difference in heights between infected and non-infected seedlings was lacking ($p \leqslant 0.1$). The mean height for non-infected seedlings was significantly lower ($p \leqslant 0.05$) during the May 1990 sample. However, this sample included several non-infected seedlings which had been browsed by deer or elk, thus reducing their total heights.

Herbivore damage accounted for 36% of the seedling mortality, while improper planting was responsible for 18%. *Fusarium* was assayed from only 18% of the dead seedlings when mortality was not traced to herbivores or poor planting. It is possible these fungi were either directly or indirectly responsible for seedling death; however, an even greater percentage (27%) died without mortality being traced to poor planting, herbivores, or *Fusarium*. These results indicate *Fusarium* spp. carried on roots of container-grown Douglas-fir seedlings are probably not a major cause of mortality once seedlings are outplanted.

Management implications

Fusarium root disease continues to be an important problem in the production of container-grown seedlings. Because fungicides fail to effectively reduce levels of *Fusarium* root infection (James et al. 1988), and quality crops can be grown with few chemical applications (Dumroese et al. 1990), an integrated pest management approach to control *Fusarium* should reduce infection and disease (James et al. 1990). Our results indicate low levels (10—40%) of root infection by *Fusarium*, mainly *F. oxysporum* and *F. proliferatum*, on Douglas-fir seedlings have little impact on outplanting performance.

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