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FUSARIUM PROLIFERATUM ROOT DISEASE ON CONTAINER-GROWN DOUGLAS-FIR SEEDLINGS FROM PACIFIC NORTHWEST REGION SEEDLOTS - USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

R.L. JAMES
Plant Pathologist*

and

R. PEREZ
Biological Technician**

ABSTRACT

Fusarium proliferatum caused extensive root disease on young container-grown Douglas-fir seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Affected seedlots were from the Malheur and Umatilla National Forests (Pacific Northwest Region). The pathogenic fungus was recovered from more than 97% of the seedlings sampled and colonized root systems at rates averaging 97%. Isolates of *F. proliferatum* were separated into four morphology types based on cultural morphology and pigment production. Morphology type was not related to virulence on Douglas-fir seedlings. Because this pathogen was isolated at very high levels from young seedlings, it is suspected that it was introduced primarily on contaminated seed. Fungicide applications did not adequately control the disease.

*Stationed in Coeur d'Alene, Idaho
**Universidad Autonoma de Neuvo Leon, Linares, Nuevo Leon, Mexico

INTRODUCTION

During the 1997 growing season, a crop of container-grown Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seedlings was produced at the USDA Forest Service Nursery, Coeur d' Alene, Idaho, for planting in the spring of 1998. This crop was grown from several seedlots collected from national forests in the Pacific Northwest Region (Region 6) in new 2A styroblock containers with operational peat/wood fiber growing media; a standard Douglas-fir growing regime was used.

Five of the seedlots (table 1) performed poorly. Seedling emergence was less than expected, resulting in many empty cavities (figure 1). Many emerged seedlings did not grow well; diseases symptoms were evident following seedling emergence and continued for many weeks. Seedlings with disease symptoms displayed needle tip necrosis (figure 2) which often progressed, resulting in seedling death. Many seedlings also became chlorotic and were stunted. These symptoms were classic for root disease on container seedlings caused by *Fusarium* spp. (James 1985; James et al. 1987). Although the crop was treated several times with topical applications of fungicides (Cleary's 3336 and iprodione applied at 10 day intervals), losses continued to occur. As a result, seedling production from these lots was less than expected and cull rates were high. An evaluation was conducted to determine possible causes of seedling mortality.

MATERIALS AND METHODS

Seventy-six seedlings with various levels of root disease symptoms (primarily needle tip necrosis and foliar chlorosis) were collected in late July; the crop was about 1.5 months old. Collected seedlings were taken to the laboratory where they were analyzed for presence of potentially-pathogenic fungi on their roots using standard isolation techniques. Roots were washed thoroughly under tap water to remove adhering particles of growing media. Root systems were then dissected into 5-8 pieces, each about 5 mm in length. Root pieces were surface sterilized in a 10% bleach (0.525% aqueous sodium hypochlorite) solution, rinsed in sterile, distilled water, and placed on an agar medium selective for *Fusarium* and closely-related fungi (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-10 days. Fungi emerging from root pieces thought to be *Fusarium* spp. were catagorized into morphology types based on superficial growth on Komada's medium. Selected representative isolates from each morphological type were single-spored and transferred to potato dextrose agar and carnation leaf agar (Fisher et al. 1982) for identification. The taxon-

Table 1. Douglas-fir seedlots from the Pacific Northwest Region severely affected with *Fusarium* root disease at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Seedlot	National Forest	Ranger District	Source Code
0491-23	Malheur	Prairie City	202-04-04076-100-5565-91 2B
0491-24	Malheur	Prairie City	202-04-04076-500-5565-91 SB
0495-42	Malheur	Long Creek	202-04-04086-500-5565-95 SB
1495-12	Umatilla	John Day	202-14-14015-505-5055-95 SB
1495-15	Umatilla	John Day	202-14-14015-105-4550-93 SIA

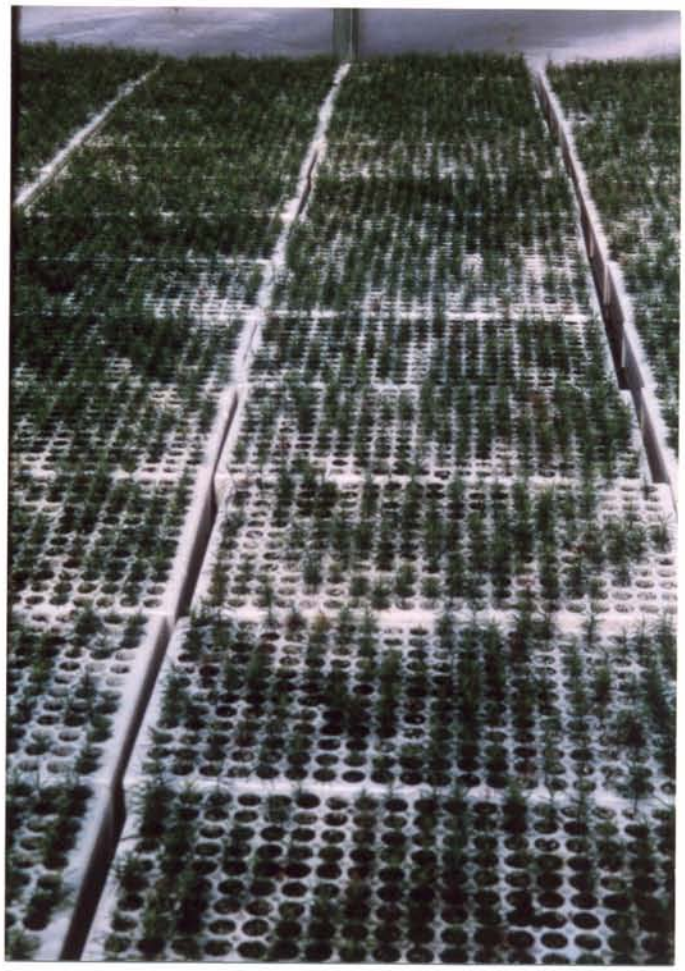

Figure 1. Container-grown Douglas-fir seedlings from Pacific Northwest Region seedlots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Note the presence of many empty cavities without seedlings.

Figure 2. Root disease symptoms on container-grown Douglas-fir seedlings from Pacific Northwest Region seedlots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Diseased seedlings (arrows) displayed needle tip necrosis.

omic scheme of Nelson et al. (1983) was used for identification of *Fusarium* species. Colonization by morphology types was summarized as percent seedlings infected and percent colonization based on number of root pieces colonized.

RESULTS AND DISCUSSION

The most commoly isolated fungus from roots of diseased Douglas-fir seedlings was *Fusarium proliferatum* (Matsushima) Nirenberg. This species was isolated from all but 2 of the seedlings (97.4 %). These two seedlings were infected with *F. oxysporum* Schlecht. Four morphology types of *F. proliferatum* were differentiated on Komada's medium. They differed in extent of aerial mycelium and level of violet pigmentation. All morphology types were classified as *F. proliferatum* primarily based on production of chains of microconidia from both mono- and polyphialides and lack of chlamydospores (Nelson et al. 1983). Root colonization percentages of the four morphology types (designated morphotypes 1-4) ranged from 4.6-60.6%; average root colonization by all *F. proliferatum* isolates was 97.0% (table 2).

Fusarium proliferatum has previously been implicated as an important cause of root disease of seedlings, especially Douglas-fir, at container nurseries (James 1997; James et al. 1991, 1995, 1997). However, most often *F. proliferatum* causes disease later in the seedling growth cycle, especially when seedlings are stressed to stop growth and set buds (James 1997; James et al. 1991). *Fusarium proliferatum* usually does not cause extensive pre- or post-emergence damping-off. (James et al. 1987). rather, *F. oxysporum*, which is often seed-borne on conifers (James 1987; James et al. 1991), is more common as a disease agent early in the container crop cycle (James 1985; James et al. 1987).

Because Douglas-fir disease severity was related to specific seedlots, pathogenic fungi were probably introduced on seed. However, seed from affected seedlots were not sampled to verify if *F. proliferatum* was introduced at high levels on them. Since *F. proliferatum* was so often isolated from young diseased seedlings, we suspect it might have been seedborne. This fungus commonly forms long chains of microconidia; when dry spores are easily disseminated in wind currents or water (James 1997; Nelson et al. 1983). It is possible that the fungus spread throughout

Table 2. *Fusarium* colonization of container-grown Douglas-fir seedling roots from Pacific Northwest Region seedlots at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fusarium spp./Morphotype	Percent Seedling Infection[1]	Percent Root Colonization[2]
F. proliferatum/Morphotype 1	75.0	60.6
F. proliferatum/Morphotype 2	51.3	33.1
F. proliferatum/Morphotype 3	25.0	18.3
F. proliferatum/Morphotype 4	5.3	4.6
All *F. proliferatum*	97.4	97.0
F. oxysporum	3.9	3.0

[1] A total of 76 seedlings with root disease symptoms were sampled.
[2] Based on percent of root pieces (5-8 sampled per seedling) colonized with appropriate fungus.

affected seedlots during processing or stratification. Perhaps only a few seeds were intially infected.

Occurrence of *F. proliferatum* isolates into different morphology types may not necessarily be related to level of virulence on Douglas-fir seedlings (James et al. 1987). Characteristics used for morphotype designation may not be genetically linked with pathogenic behavior. Experience indicates that most isolates of *F. proliferatum* are very virulent to Douglas-fir when tested in laboratory pathogenicity assays (James et al. 1997). Therefore, we suspect that most of the *F. proliferatum* isolates obtained from these container-grown seedlings were virulent and aggressive pathogens, even though they appeared morphologically different.

Use of fungicides to control this disease was disappointing. Past experience indicates that root disease caused by *F. proliferatum* cannot routinely be sucessfully controlled by fungicides once disease symptoms appear (James et al. 1987, 1991). Because the fungus usually affected seedlings late in the growth cycle, it was thought that fungitoxic chemicals could not reach sites of root infection at high enough concentrations to be effective (James et al. 1987). However, it is possible that some inherent resistance occurs in *F. proliferatum* to standard fungicides. This could be tested by evaluating representative isolates for their resistance to common fungicides using *in vitro* tests on agar amended with fungicides. Such techniques have been effective in evaluating resistance of *Botrytis cinerea* Pers. ex. Fr. to certain fungicides (James and Gilligan 1985).

We conclude that the primary cause of poor seedling establishment and root disease of Douglas-fir from certain Pacific Northwest Region seedlots was due to widespread occurrence and damage by *F. proliferatum*. It is likely that this pathogen was introduced on seed. If these seedlots are to be used to produce future seedling crops, it is recommended that they be evaluated for level of *Fusarium* contamination. If these pathogens are found at high levels, pre-stratification seed surface sterilization treatments are recommended.

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