FUNGAL COLONIZATION OF DOUGLAS-FIR SEED AND CONTAINER-GROWN SEEDLINGS FROM THE NORTH WOODS NURSERY, ELK RIVER, IDAHO

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Nursery Disease Notes #99

January 1990

During production of the 1989 crop of container-grown Douglas-fir (**Pseudotsuga menziesii** (Mirb.)Franco) seedlings at the North Woods Nursery (Elk River, Idaho), root disease symptoms were noticed on seedlings within seedlot 87-50. Disease levels were not extensive and only a few diseased seedlings were found in each styroblock tray. The affected seedlot which was obtained from Potlatch Corporation (Lewiston, ID) had been processed by the Brown Seed Company (Vancouver, WA). Growers were interested to know if seed contaminated with pathogenic fungi might be a major source of root disease inoculum.

Samples collected from the nursery included ungerminated seed that had been sown, seedlings with root disease symptoms, and bulk seed from storage. In addition, another seed sample from the same seedlot having been processed and stored at the Brown Seed Company was also analyzed. Sample seed were aseptically placed directly on an agar medium selective for **Fusarium** spp. and related root disease fungi (Komada 1975). In most cases, 15 seeds were placed on each 90mm plate containing about 15 ml of agar. Roots of sampled seedlings were washed thoroughly under running tap water to remove adhering growth medium particles. Roots were then cut into 3-5mm pieces, surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite) for 1 minute, rinsed with sterile distilled water, and placed on the selective agar medium. All plates were incubated under diurnal cycles of cool fluorescent light at about 26°C for 7-10 days. Fungi emerging from seed or seedling roots were identified to genus using a standard taxonomic guide (Barnett and Hunter 1972). Representative **Fusarium** spp. were transferred to potato dextrose and carnation leaf agar to facilitate identification using monographs of the genus (Gerlach and Nirenberg 1982; Nelson and others 1983).

Extent of fungal colonization of the different seed samples is summarized in table 1. **Fusarium** spp. were found on all sown seed (sample set 1) and on more than 70% of the bulk seed from the North Woods Nursery (sample set 2). However, only about 13% of the seed sent from the Brown Seed Company (sample set 3) were colonized with **Fusarium** spp., although all sampled seed were from the same lot. The most common **Fusarium** species isolated from seed from the North Woods Nursery was **F. oxysporum** Schlect., whereas those species isolated from seed from the Brown Seed Company were a combination of **F. acuminatum** Ell. & Ev., **F. sambucinum** Fuckel, and **F. equiseti** (Corda) Sacc. Although **Fusarium** spp. can sometimes be found within the seed

embryo (James 1984b, 1986, 1987), most propagules were probably located externally on the seedcoat. The disparity in **Fusarium** levels between bulk seed samples from the nursery and Brown Seed Company is difficult to explain. It is possible that during transport, handling or stratification, seed became contaminated with **F. oxysporum** and this fungus spread throughout much of the seedlot. The fact that very little of this fungal species was found on processed seed from the Brown Seed Company (table 1) would indicate that spread of the pathogen among seed probably occurred after shipment. Even though **Fusarium** spp. are usually considered relatively inactive at low temperatures (Nelson and others 1983), they have been shown to spread and contaminate seed during stratification (W. Littke, personal communication). This may have occurred with seedlot 87-50 at the North Woods Nursery.

Fusarium spp. isolated from roots of diseased seedlings (table 2) were the same species that were isolated from seed with the exception of **F. equiseti**, which was not found on seedling roots. **Fusarium acuminatum** was the most frequently isolated species from seedling roots. This fungus is often associated with root diseased Douglas-fir seedlings (James and others 1988b) and is capable of eliciting disease symptoms in inoculated hosts (James and others 1988a). **Fusarium oxysporum** and **F. sambucinum** were isolated at about the same frequency (colonization intensity) from the roots of diseased seedlings (table 2). Although both species are frequently associated with root diseased Douglas-fir seedlings (James and others 1988b), **F. oxysporum** is usually a much more aggressive pathogen (James and Gilligan 1984; James and others 1988a).

Other fungi isolated from Douglas-fir seedlot 87-50 included **Cylindrocarpon** sp., **Trichoderma** spp., **Penicillium** spp., **Phoma herbarum** Westend., and **Botrytis cinerea** Pers:.Fr. (table 1). By far the most common fungi, other than **Fusarium**, were **Trichoderma** and **Penicillium**, both of which are usually saprophytes. **Trichoderma** may be competitive with or antagonistic toward **Fusarium** (Papavizas 1985). **Trichoderma** are often isolated at higher frequency when **Fusarium** spp. are found at low levels and vice versa (James and others 1987). This occurred with isolations from Douglas-fir lot 87-50, i. e. higher **Trichoderma** levels were found in sample set 3 (Brown Seed Company) where lower amounts of **Fusarium** were isolated.

Cylindrocarpon spp., **Phoma herbarum**, and **Botrytis cinerea**, all potential conifer seedling pathogens (James 1984a, 1985, 1988), were found at very low levels. Neither of these organisms were likely responsible for eliciting disease of Douglas-fir seedlings at the North Woods Nursery.

In conclusion, **Fusarium** spp. (most likely a combination of **F. oxysporum** and **F. acuminatum**) were responsible for the small amount of root disease found on Douglas-fir seedlings from lot 87-50. Even though **F. oxysporum** contaminated a large percentage of sown seed, disease levels were quite low. This fungus may readily colonize the roots of container-grown seedlings without eliciting disease symptoms (James and others 1987; James and Gilligan 1988). Factors contributing to disease sysmptom production of infected seedlings are largely

Table 1. Colonization of Douglas-fir seed (lot 87-50) from the North Woods Nursery with selected fungi.

Fungi Assayed	Colonization Percentage Sample Set[1] 1	2	3
Fusarium oxysporum	80	70.6	0.3
Fusarium "roseum"[2]	60	2.6	13.0
All **Fusarium**	100	71.3	13.3
Cylindrocarpon sp.	0	0.0	0.3
Trichoderma spp.	0	37.5	74.3
Penicillium spp.	0	60.6	67.7
Phoma herbarum	0	1.2	0.3
Botrytis cinerea	0	0.0	4.3

[1] Set 1: from sown seed (sample size = 5).
Set 2: from bulk (unsown) seed from the North Woods Nursery (sample size = 160).
Set 3: from bulk (processed) seed from the Brown Seed Company (sample size = 300).

[2] Comprised of three species: **Fusarium acuminatum**, **F. sambucinum**, and **F. equiseti**.

Table 2. Colonization of roots of diseased container-grown Douglas-fir seedlings with **Fusarium** spp. at the North Woods Nursery, Elk River, Idaho.

Fusarium species	Percentage Colonization Seedlings*	Intensity**
F. oxysporum	100	39.0
F. acuminatum	100	56.1
F. sambucinum	40	38.9
All species	100	100.0

* Percentage of sampled seedlings (sample size = 36) colonized with appropriate fungi.
** Percentage of sampled root pieces colonized with appropriate fungus.

unknown, but may involve microbial interactions including antagonism by other fungi, levels of virulence of associated fusaria, and interactions with environmental factors such as temperature and moisture. In any event, levels of Douglas-fir root disease were not well correlated with levels of **Fusarium** found on seed from the North Woods Nursery.

Root disease levels can be reduced by rinsing seed with running water for at least 48 hours prior to sowing. This will not only condition seed for rapid germination, but also reduces levels of pathogenic fungi residing on seedcoats (James 1987). If disease is found early in the life of the crop, fungicide drenches may be applied to restrict development of further disease. However, such drenches are usually not very effective during later stages of crop development (James and others 1988b).

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