FUNGAL COLONIZATION OF ROOTS FROM BAREROOT 2-0 DOUGLAS-FIR SEEDLINGS AT THE USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

R. L. James
Plant Pathologist

USDA Forest Service
Northern Region
1201 Ironwood Drive
Coeur d'Alene, ID 83814

Nursery Disease Notes #86

May 1989

During investigations of pathogenic fungi associated with roots of conifer seedlings, techniques are used to isolate organisms that are colonizing the roots. Usually, these techniques involve washing roots to remove adhering soil particles and attempting to sterilize root surfaces to exclude those fungi that are residing on the root surface (rhizosphere). The goal is to limit isolations to only those organisms that are actually colonizing cortical root tissues so that only those fungi that are responsible for or capable of causing disease symptoms are obtained. Likewise, it is desirable that those organisms which are saprophytic colonizers of organic matter or root surfaces are excluded. These procedures are complicated by the fact that many fungi which reside on root surfaces are also capable of colonizing cortical cells and may be either parasitic or saprophytic. Therefore, it is often not possible to eliminate probable saprophytes on the basis of fungal identity alone. However, if surface contaminating fungi can be reliably eliminated during isolation work, remaining fungi can theoretically be regarded as true colonizers of cortical cells and more probable pathogens. True pathogenic potential of fungi can only be evaluated using carefully controlled inoculation tests on susceptible host plants.

An investigation was conducted to evaluate techniques of root surface sterilization to determine common fungal colonizers of cortical cells. Comparisons were made among the different treatments to determine differences in seedling root mycofloras. Bareroot 2-0 Douglas-fir (Pseudotsuga menziesii (Mirb.)Franco) seedlings without disease symptoms were selected for analysis to determine background levels and identity of fungal organisms colonizing cortical tissues.

Root systems of fifty recently-lifted seedlings were placed in standing water for 48 hours. Selected roots were severed from seedlings and washed for

several minutes under running tap water to remove soil. Pieces of root were randomly selected from the composite of root systems and cut to approximately 0.5 cm in length. Root pieces were selected from throughout root systems, including terminal as well as intercalary locations. Ten pieces were subjected to each surface sterilization treatment (table 1). Following treatment, root pieces were rinsed thoroughly in sterile distilled water and aseptically placed on a selective agar medium commonly used to isolate pathogens (Komada 1975). Controls consisted of placing untreated (washed) root pieces on the selective medium. Plates with root pieces were incubated under diurnal cycles of cool fluorescent light at about 22-24°C for 7-10 days. Emerging fungi were transferred to potato dextrose agar (PDA) slants and identified using standard taxonomic guides (Barnett and Hunter 1972; Nelson, Toussoun and Marasas 1983).

Table 1. Surface sterilization procedures tested on roots of bareroot 2-0 Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Treatment	Description
A-1	Immersed in 10% bleach[1] for 30 seconds.
A-2	Immersed in 10% bleach for 60 seconds.
A-3	Immersed in 10% bleach for 120 seconds.
A-4	Immersed in 10% bleach for 240 seconds.
B-1	Immersed in 50% bleach[2] for 30 seconds.
B-2	Immersed in 50% bleach for 60 seconds.
B-3	Immersed in 50% bleach for 120 seconds.
B-4	Immersed in 50% bleach for 240 seconds.
C-1	Immersed in 100% bleach[3] for 30 seconds.
C-2	Immersed in 100% bleach for 60 seconds.
C-3	Immersed in 100% bleach for 120 seconds.
C-4	Immersed in 100% bleach for 240 seconds.
D-1	Immersed in 95% ethanol for 10 seconds.
D-2	Immersed in 95% ethanol for 30 seconds.
E-1	Immersed in 95% ethanol for 5 seconds and flamed.

[1] 0.525 percent aqueous sodium hypochlorite.

[2] 2.625 percent aqueous sodium hypochlorite.

[3] 5.250 percent aqueous sodium hypochlorite.

Effects of surface sterilization treatments on recovery of fungi from Douglas-fir seedling roots are summarized in table 2. Five groups of fungi which could be readily identified on the selective medium (Fusarium, Cylindrocarpon, Trichoderma, Penicillium, and Phoma) were assayed. All unidentified bacterial colonizers were grouped together. Root pieces from which neither fungi nor bacteria were isolated were designated "clean".

Table 2. Effects of root surface sterilization on recovery of fungi from the roots of bareroot 2-0 Douglas-fir seedlings-USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Treatment[1]	Percent Root Pieces Colonized						
	Fusarium	Cylindrocarpon	Trichoderma	Penicillium	Phoma	Bact.	Clean[2]
A-1	0	0	90	0	60	20	0
A-2	10	0	50	0	70	10	0
A-3	0	0	20	0	90	20	0
A-4	0	0	10	0	70	20	0
B-1	0	0	10	0	60	0	30
B-2	0	0	0	10	40	50	0
B-3	0	0	20	0	70	10	10
B-4	0	0	10	10	80	20	0
C-1	0	0	40	0	50	20	10
C-2	0	0	0	0	50	10	40
C-3	0	0	20	0	50	10	20
C-4	10	0	10	0	20	0	60
D-1	0	0	70	0	0	30	0
D-2	0	0	40	0	30	30	10
E-1	0	0	0	0	0	0	100
Control	0	20	85	0	0	80	0

[1] See table 1 for treatment descriptions.

[2] Without fungal or bacterial colonization.

The two major groups of fungi consistently isolated from roots were Trichoderma spp. and Phoma spp.. Exposure to bleach at higher concentrations or for longer time periods generally reduced amounts of Trichoderma recovered. Apparently, Trichoderma spp. consistently occupied the rhizosphere or outer cortical cells and were sensitive to surface sterilization treatments. However, no consistent treatment effects on the recovery of Phoma spp. were found. These fungi apparently colonized cortical tissues and/or were not sensitive to the surface sterilization treatments. Recent attempts to clean containers used to grow conifer seedlings (James and Woolen 1989; Sturrock and Dennis 1988) have indicated that Phoma spp. are often very difficult to remove from containers treated with sterilants and hot water. Apparently, these fungi are less sensitive to bleach and certain other sterilants than other fungi.

All isolates of Phoma obtained from Douglas-fir roots were quite similar. They produced an olivaceous-grey aerial mycelium on PDA and pycnidia embedded within the agar or directly on the colony surface. These isolates were probably either P. eupyrena Sacc. or closely related species (Dorenbosch 1970). These Phoma spp. were probably common nursery soil inhabitants. Although Phoma spp. are capable of causing seedling diseases (James and Hamm 1985), those isolated were most likely saprophytic colonizers of root cortical tissues.

Fusarium spp. were recovered from only two of the root pieces assayed in this investigation, even though they may commonly colonize roots of non-diseased bareroot Douglas-fir seedlings (James and Gilligan 1988). One of the root pieces was colonized with F. oxysporum Schlect., while the other was infected with F. acuminatum Ell. & Ev. Although both species may be pathogenic to Douglas-fir seedlings (James et al. 1989), saprophytic strains also occur. Since none of the evaluated seedlings displayed typical root disease symptoms, it is likely that these strains were either saprophytic or at insufficient concentrations within host tissues to elicit disease symptoms (Harling et al. 1988).

Cylindrocarpon spp., close relatives of Fusarium spp. and potential pathogens of conifer seedlings (Booth 1966), were only isolated from control (untreated) roots. These fungi were not important colonizers of the roots examined in this evaluation.

This evaluation indicated that exposure to a 10% bleach solution for about 2 min. (or higher concentrations for less time) was effective in reducing root surface colonization by Trichoderma and possibly other fungi which were not assayed. Surface sterilizing with a 100% bleach solution, especially for 4 min., resulted in a large percentage of root pieces which were not colonized with any fungi. It is likely that the bleach penetrated cortical cells and was toxic to fungi residing there. Root exposure to 95% ethanol for 30 sec. was also effective in reducing fungi. Flaming tissues treated with ethanol eliminated all fungal colonizers.

In conclusion, this evaluation has shown that fungal colonization of Douglas-fir seedling root cortical tissues is very common, even though infected seedlings appear healthy. Root surface fungi can adequately be eliminated by treatment with either bleach or ethanol. Exposure of roots for longer periods of time or to higher sterilant concentrations results in killing fungi within the cortical cells.

LITERATURE CITED

Barnett, H. L. and B. B. Hunter. 1972. Illustrated genera of imperfect fungi. Burgess Publ. Co., Minneapolis, MN. 241p.

Booth, C. 1966. The genus Cylindrocarpon. Commonwealth Mycological Institute, Mycological Papers No. 14. 56p.

Dorenbosch, M. M. J. 1970. Key to nine ubiquitous soil-borne Phoma-like fungi. Personnia 6(1):1-14.

Harling, R., G. S. Taylor, P. Matthews, and A. E. Arthur. 1988. The effect of temperature on symptom expression and colonization in resistant and susceptible carnation cultivars infected with Fusarium oxysporum f. sp. dianthi. Journal of Phytopathology 121:103-117.

James, R. L., R. K. Dumroese, C. J. Gilligan and D. L. Wenny. 1989. Pathogenicity of Fusarium isolates from Douglas-fir seed and container seedlings. University of Idaho. For., Wildlife and Range Exp. Sta. Rept. (In press).

James, R. L. and C. J. Gilligan. 1988. Occurrence of Fusarium on the roots of nondiseased bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Rept. 88-12. 4p.

James, R. L. and P. B. Hamm. 1985. Chlamydospore-producing species of Phoma from conifer seedlings in Pacific Northwest forest tree nurseries. Proc. Montana Acad. Sci. 45:26-36.

James, R. L. and R. L. Woolen. 1989. An evaluation of the efficacy of hot water-chemical treatments to clean styroblock containers - Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region (In press).

Komada, H. 1975. Development of a selective medium for quantitative isolation of Fusarium oxysporum from natural soil. Rev. Plant Prot. Res. 8:114-125.

Nelson, P. E., T. A. Toussoun and W. F. O. Marasas. 1983. Fusarium species: and illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

Sturrock, R. N. and J. J. Dennis. 1988. Styroblock sanitation: results of laboratory assays from trials at several British Columbia forest nurseries. In: Landis, T. D. (tech. coord.). Proceedings: Combined Meeting of the Western Forest Nursery Associations. USDA Forest Service, Gen. Tech. Rept. RM-167. pp. 149-154.