NURSERY DISEASE NOTES USDA FOREST SERVICE

NORTHERN REGION
FOREST HEALTH PROTECTION

No. 135 March 1998

QUANTIFICATION OF CONIFER SEEDLING ROOT COLONIZATION BY *FUSARIUM* AND *CYLINDROCARPON* SPECIES

R. L. JAMES
Plant Pathologist*

ABSTRACT

An evaluation was conducted to determine accuracy of a standard root tip analysis for estimating level of root colonization by the potential pathogenic fungi *Fusarium* and *Cylindrocarpon*. Colonization intensities were calculated from incubating several lengths of root (at least 100 cm per seedling); these were compared with intensities and percentages calculated from incubating 10 small root pieces per seedling. Mean colonization rates were calcuated to estimate the probability of rhizosphere *Fusarium* propagules to infect adjacent roots. Rates were calculated as the number of *Fusarium* colonies per 100 cm of roots divided by the average number of colony-forming units of *Fusarium* in the rhizosphere. Root tip assay methods were not good predictors of total root colonization intensity. Although quick assay of root tips can be useful to determine presence or absence of potentially-pathogenic fungi on conifer seedling root systems, they do not accuately estimate level of root colonization. For accurate root colonization estimates, much more extensive root system assay is required.

INTRODUCTION

Fusarium species are common fungal colonizers of container and bareroot conifer seedling roots in forest nurseries. These fungi are often isolated from roots of both diseased and healthy-appearing seedlings (James et al. 1991). They are also common residents of forest nursery soil and have traditionally been controlled by fumigation with general biocides such as methyl bromide/chloropicrin (James 1989). However, methyl bromide is currently being phased out as a soil fumigant because it is an important destroyer of stratospheric ozone (Stone et. al.

* Stationed in Coeur d'Alene, Idaho

1997). Therefore, within the United States, methyl bromide will not longer be manufactured or used after Jan. 1, 2001.

Efforts in the western United States have recently concentrated on developing strategies for growing bareroot seedlings without soil fumigation. This will require improved integrated pest management techniques for determining levels of potential pathogenic fungi within nursery soil and on the seedling roots. Improved monitoring of potential pathogens will be required to enhance efficacy of suppression efforts. Standard soil dilution methods are available that give rough estimates of fungal soil populations in the genera *Fusarium* and *Pythium*. However, estimating levels of root colonization by these fungi is often more difficult.

Past efforts at estimating levels of root colonization by *Fusarium* and other potential plant-pathogenic fungi have often involved selecting a minimum of 10 root pieces per seedling for isolation on selective agar media. These pieces are either excised from the tips of roots or randomly selected from throughout the root system. Roots are initially washed to remove most adhering soil or growing media particles. Selected root pieces are usually surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite) and rinsed in sterile distilled water prior to placement on agar media. Colonization percentages are recorded as the percentage of root pieces on which a particular fungus is located. This sampling procedure is quick, but may not accurately reflect actual root colonization.

A procedure for evaluating *Fusarium* colonization of muskmelon plants was recently reported by Gordon et al. (1989). This technique involved evaluation soil populations of selected *Fusarium* species and formae speciales near infected plants, determining the number of *Fusarium* infections along lengths of roots and calculating a value called "mean colonization rate" based on these two assays. This rate gives an indication of how much root infection occurred relative to propagule density in nearby soil. That way plant infection predictions (and perhaps disease levels) could be determined based on known levels of soil populations.

The above procedure has not been evaluated for root colonization of bareroot conifer seedlings in nurseries. Therefore, an evaluation was conducted to determine if a similar procedure would be of value in estimating relative levels of root colonization and, coupled with data on soil populations, be accurate in predicting root colonization from soil populations.

MATERIAL AND METHODS

This evaluation was conducted at the USDA Forest Service Nursery in Coeur d'Alene, Idaho in an area previously used for evaluating alternatives to soil fumigation. At the end of a two-year growing cycle, Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seedlings were lifted according to normal nursery practice. Seedlings within plots that had been untreated (fallowed prior to planting with no fumigation) were mostly healthy without root disease symptoms. Fifty seedlings from these plots were randomly collected and taken to the laboratory for analysis.

Seedling roots were gently washed to remove rhizosphere soil, which was collected from each root system. The soil was dried for several days on lab benches and samples from all fifty seedlings collated. The collated sample was then sub-divided into 25 individual samples. Standard soil dilutions were conducted on the individual samples. In this procedure, 0.5g of each sample was mixed with 100 ml of 0.3% water agar; 1 ml of the solution was placed on each of 5 plates of a selective agar medium for *Fusarium* and associated fungi (Komada 1975). Plates were incubated under cool, fluorescent

light at about 24°C for 7 days after which colonies of *Fusarium* were counted. Colonies were divided into morphology types and representative samples of each morphology type transferred to potato dextrose and carnation leaf agar (Fisher et al. 1982) for identification of individual *Fusarium* species comprising each morphology type. Populations (colony-forming units/g) of each *Fusarium* species were calcuated for each of the 25 samples. Averages were determined and used in other calculations.

After washing, ten root pieces, each about 0.5 cm in length, were excised from the tips of randomly-selected roots, surface sterilized with bleach (0.525% aqueous sodium hypochlorite) and placed on Komada's medium. This procedure of estimating level of root colonization from sampling root tips has been used in several previous evaluations, especially those in which destructive samples of root systems could not be taken (Dumroese et al. 1993, 1995) and is referred to as the **root tip method**. The number of root pieces colonized with *Fusarium* and/or *Cylindrocarpon* was determined. Colonization percentages were calculated as the number of root tips colonized multiplied by 10.

For comparisons, another method (**root length method**) involved randomly selecting several lengths of seedling roots, excising them and placing them directly on the surface of Komada's medium after standard surface sterilization. A minimum of 100 cm of roots from each seedling was assayed in this method; it took several plates of media per seedling. Plates were incubated as described above and the number of *Fusarium* and *Cylindrocarpon* colonies growing from roots were determined. Colonies were again divided into morphology types so that the occurrence of individual *Fusarium* species on roots could be determined.

Colonization intensity was calculated as the average number of colonies of *Fusarium* and *Cylindrocarpon* isolated from 10 cm of root tissue. To estimate the number of colonies formed on root tip pieces (**colonization intensity-tips**), it was assumed that each colonized piece (0.5 cm in length) was infected with one colony. Colonization intensity was determined for the root tip method by assigning one colony per 0.5 cm colonized piece of root tip. For example, if 3 of the 10 root tips were colonized with *Fusarium*, the colonization intensity would be 6.0 (3 colonies per 5 cm multiplied by 2 for the number per 10 cm; the maximum colonization intensity was 20.0 if all root tip pieces were colonized). Colonization intensity for the root length method (**colonization intensity-lengths**) was calculated directly as the average number of colonies per 10 cm of root.

Mean colonization rate of *Fusarium* was calculated for each seedling using the technique described by Gordon et al. 1989, 1990. This rate estimated the probability of rhizosphere *Fusarium* propagules to infect adjacent roots and was calculated as the number of *Fusarium* colonies per 100 cm of roots divided by the average number of colony-forming units of *Fusarium* in the rhizosphere. Using this procedure an approximation of expected root colonization could be estimated from sampled soil populations. Individual mean colonization rate averages were calculated for all the sampled seedlings and an average rate determined. Rates were determined for *Fusarium oxysporum*, and grouped for other *Fusarium* species and all *Fusarium* species.

Colonization rates, intensities and percentages were summarized as averages for the 50 seedlings. Standard deviations and variances were calculated. Simple linear regressions were conducted comparing percent colonization via the root tip method (independent variable) with colonization intensities-tips and lengths (dependent variables). Coefficients of determination (r^2) were determined from each regression.

RESULTS AND DISCUSSION

Fusarium spp. were common colonizers of rhizosphere soil from Douglas-fir seedling roots and were found in all 25 samples. Average population for all *Fusarium* spp. was about 243 cfu/g (table 1). The population comprised five different *Fusarium* species, including *F. oxysporum* Schlecht., *F. acuminatum* Ell. & Ev., *F. sporotrichioides* Sherb., *F. avenaceum* (Fr.) Sacc., and *F. solani* (Mart.) Appel & Wollenw. By far the most common species isolated from rhizosphere soil was *F. oxysporum*, comprising about 83% of the *Fusarium* population. *Trichoderma* spp., common soil colonizers and potentially antagonistic toward *Fusarium* spp. and other plant pathogens (Papavizas 1985), were also assayed from rhizosphere soil (table 1). Levels of these fungi exceeded *Fusarium*. The overall *Trichoderma/Fusarium* ratio for rhizosphere soil was 2.84; this rate gives a rough estimate of the potential suppressiveness of the soil to *Fusarium* caused by *Trichoderma* spp. The higher the ratio, the more suppressive the soil to pathogenic fungi.

Comparisons of the various colonization estimation methods for *Fusarium* spp. are summarized in table 2. The typical root tip colonization method for all *Fusarium* isolates gave an overall average colonization of 26%. If this information was used to calculate the colonization intensity (tips), the average intensity was 5.2. This value was larger than the colonization intensity

Table 1. Populations of *Fusarium* and *Trichoderma* spp. in the rhizosphere of nondiseased bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fungal Species	Average Colony-Forming Units/g of Soil[1]
Trichoderma spp.	691.1 (±359.9)
All *Fusarium* species	242.9 (±133.7)
Fusarium oxysporum	202.2 (±135.2)
Fusarium acuminatum	27.9 (±41.4)
Fusarium sporotrichioides	8.2 (±23.2)
Fusarium avenaceum	3.3 (±16.1)
Fusarium solani	1.6 (±8.0)

[1] Based on 25 soil samples collected from the rhizosphere of 50 seedlings. Standard deviations are in parentheses.

calculated from sampling entire roots. Colonization intensity-lengths averaged 3.1, an 40% decrease from that estimated from root tips. *Fusarium oxysporum* was by far the most common *Fusarium* species isolated from seedling roots, making up more than 93% of all colonies. Other *Fusarium* species colonizing roots included *F. solani, F.acuminatum, F.avenaceum, F. sambucinum, F.poae* (Peck.) Wollenw., and *F. equiseti* (Corda) Sacc. Regressions to predict overall root colonization from percent of root tip colonization resulted in coeffcients of determination of 0.200 for colonization intensity-tips and 0.046 for colonization intensity-lengths. Such poor correlation indicated that accurate *Fusarium* root colonization could not be predicted from percent root tip colonization.

Mean colonization rates for *Fusarium* averaged 0.1263 for all species (table 2). This indicated that there was a 12.6% probability of each *Fusarium* propagule occuring in the rhizosphere colonizing a nearby root. The probability was somewhat higher for *F. oxysporum* (0.1441), indicating that this species was more likely to colonize seedling root tissues than some of the other *Fusarium* species which had much lower mean colonization rates (average for all other *Fusarium* species = 0.0382). Gordon et al. (1989) found rates of 0.140 for non-pathogenic *F. oxysporum* in naturally infested soil, whereas the rate for pathogenic *F. oxysporum* (*F. oxysporum* f. sp. *melonis*) was much lower (0.080)

Table 2. Comparison of root colonization estimate methods for *Fusarium* species on bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Root Colonization Assay	Average Colonization[1]		
Technique	*Fusarium oxysporum*	Other *Fusarium* Species[2]	All *Fusarium*
Percent Colonization[3]	-	-	26.0 (±17.3)
Colonization Intensity-Tips[4]	-	-	5.2 (±3.5)
Colonization Intensity-Lengths[5]	2.9 (±1.6)	0.2 (±0.2)	3.1 (±1.6)
Mean Colonization Rate[6]	0.1441 (±0.0804)	0.0382 (±0.0422)	0.1263 (±0.0660)

[1] Average colonization of 50 healthy 2-0 Douglas-fir seedlings: standard deviation in parentheses.
[2] Includes *F. solani, F. acuminatum, F. avenaceum, F. sambucinum, F. poae* and *F. equiseti.*
[3] Based on numbers of root pieces (10 sampled per seedling) colonized with appropriate fungus.
[4] Derived from percent root tip colonization - estimate of number of colonies occurring on 10 cm of root tips.
[5] Calculated as the average number of colonies per 10 cm of root (minimum of 100 cm sampled per seedling).
[6] Average number of colonies per 100 cm of roots divided by the average number of colony-forming units in the rhizosphere.

Under more natural conditions (typical field soil), colonization rates were much lower: 0.040 for non-pathogenic *F.oxsyporum* and 0.030 for pathogenic isolates. Isolates of *F.oxysporum* pathogenic or non-pathogenic to Douglas-fir seedlings cannot easily be separated since they all appear morphologically similar. However, because sampled seedlings did not exhibit typical root disease symptoms, it was suspected that a majority of the *F.oxysporum* isolates obtained from roots were non-pathogenic. If this assumption was true, then colonization rates by *F. oxysporum* from nursery soil were similar to those from naturally-infested agricultural soil. This species is a common inhabitant of forest nursery soil and may exist at relatively high population levels (James et al. 1991).

Cylindrocarpon spp. also commonly colonized Douglas-fir seedling roots. Colonization percentages (root tips) and colonization intensities were higher than those for *Fusarium* (table 3). The average colonization intensity obtained from root tip samples was again higher than that obtained from entire root lengths (11.0 vs. 7.8). This represented a 29% difference in the estimate of colonization intensity based on the two methods. Regressions to predict overall colonization intensity from root tip colonization percentages resulted in coefficients of determination of 0.200 for colonization intensity-tips and 0.011 for colonization intensity-lengths. This poor correlation indicated that accurate colonization intensity by *Cylindrocarpon* could not be predicted from root tip colonization percentages.

This evaluation confirmed that colonization of Douglas-fir seedling roots by *Fusarium* and *Cylindrocarpon* spp. is very common, even on seedlings without disease symptoms (James and Gilligan 1988). Colonization rates were quite high with potentially-pathogenic fungi occupying extensive root cortical tissues. Unfortunately, the data showed that actual root

Table 3. Comparison of root colonization estimate methods for *Cylindrocarpon* spp. on bareroot Douglas-fir seedings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Root Colonization Assay	Average Colonization by *Cylindrocarpon* spp.[1]
Percent Colonization[2]	55.2 (±22.8)
Colonization Intensity-Tips[3]	11.0 (±4.6)
Colonization Intensity-Lengths[4]	7.8 (±1.3)

[1] Average colonization of 50 healthy 2-0 Douglas-fir seedlings; standard deviation in parentheses.
[2] Based on number of root pieces (10 sampled per seedling) colonized with *Cylindrocarpon* spp.
[3] Derived from percent root tip colonization - estimated number of colonies isolated per 10 cm of root tips sampled.
[4] Calculated as the average number of colonies per 10 cm of root (minimum of 100 cm sampled per seedling).

colonization could not be accurately predicted from a small sample of root tips. Root tip colonization has apparantly over estimated actual root colonization by *Fusarium* and *Cylindrocarpon* in the past. This evaluation showed that it might be possible to estimate mean colonization rate of roots from information on *Fusarium* populations in nearby soil. However, disease intensity cannot accurately be predicted from soil populations because of the diversity of pathogenic potential in *Fusarium* soil populations. Overall presence of *Fusarium* in soil or level of root infection may not be satisfactory indications of expected disease. Further work to characterize *Fusarium* populations for potential virulence is required. Molecular genetic analysis probably provides the best potential for population characerization, particularly if genetic markers can be identified that separate pathogenic from non-pathogenic members of populations.

LITERATURE CITED

Dumroese, R.K., R.L. James and D.L. Wenny. 1993. *Fusarium* root infection of container-grown Douglas-fir: effects on survival and growth of outplanted seedlings and persistence of the pathogen. New Forests 7:143-149.

Dumroese, R.K., R.L. James and D.L. Wenny. 1995. Interactions between copper-coated containers and Fusarium root disease: a preliminary report. USDA Forest Service, Northern Region, Insect and Disease Management. Report 95-9. 8p.

Fisher, N.L., L.W. Burgess, T.A. Toussoun and P.E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151-153.

Gordon, T.R., D.J. Jacobson, D.M. May, K.B. Tyler and F.W. Zink. 1990. Fruit yield, disease incidence, and root colonization by hybrid muskmelon resistant to *Fusarium* wilt. Plant Disease 74:778-781.

Gordon, T.R., D. Okamoto and D.J. Jacobson. 1989. Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f. sp. *melonis* and other species of *Fusarium*. Phtyopathology 79:1095-1100.

James, R.L. 1989. Effects of fumigation on soil pathogens and beneficial microorganisms. *In*: Landis, T.D. (tech. coord.). Proceedings: Intermountain Forest Nursery Association Meeting. USDA Forest Service, General Technical Report RM-184. pp. 29-34.

James, R.L., R.K. Dumroese and D.L. Wenny. 1991. *Fusarium* diseases of conifer seedlings. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada, Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

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

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review Plant Protection Research (Japan) 8:114-125.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-54.

Stone,J.K., D. Hildebrand, R.L. James and S.J. Frankel. 1997. Alternatives to chemical fumigation in bareroot forest nurseries: effects on pathogen levels and seedling density, mortality and quality. *In*: James, R.L. (ed.). Proceedings of the third meeting of IUFRO Working

Party s7.03-04 (Diseases and Insects in Forest Nurseries). USDA Forest Service, Northern Region, Forest Health Protection. Report 97-4. pp. 59-69.