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FUNGAL COLONIZATION OF ROOTS FROM WESTERN WHITE PINE TRANSPLANT SEEDLINGS OUTPLANTED ON THE WALLACE RANGER DISTRICT, IDAHO PANHANDLE NATIONAL FORESTS, IDAHO

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Western white pine (*Pinus monticola* Dougl.) is one of the most important reforestation conifer species in the Inland Northwest. Recent tree improvement efforts have emphasized production of seedlings with levels of resistance to blister rust (*Cronartium ribicola* Fisch.) for outplanting on forest sites in northern Idaho and western Montana. Because of limited amounts of improved seed currently being produced in seed orchards, individual seedlings are of very high value.

Recently, some forest managers have indicated preference for transplant stock to outplant on sites where a larger seedling is required for successful establishment. Therefore, the USDA Forest Service Nursery in Coeur d'Alene, Idaho, has evaluated costs and limitations of producing transplant stock. Nursery growers evaluated both "plug + 1" (container-grown seedlings planted in bareroot beds for an additional year) and "2 + 1" (two-year old bareroot seedlings transplanted to another field at the nursery for an additional year's growth). Unfortunately, relatively high levels of mortality were encountered during this evaluation (James, unpublished). Much of the mortality was associated with extensive root infection by *Fusarium* spp. These fungi are important root pathogens of container-grown stock at the nursery (James and others 1987), but normally colonize roots of bareroot stock at lower levels (James and Gilligan 1988). During lift and pack operations, transplant seedlings with moderate to severe root disease symptoms were culled before being shipped for outplanting.

Forest managers were concerned that seedlings without root disease symptoms at the time of shipment might have been infected with root pathogens which may affect outplanting performance. Therefore, an evaluation was conducted to determine extent and characteristics of fungi colonizing transplants after outplanting.

Two plantations (designated 3G and 3H) on the Wallace Ranger District, Idaho Panhandle National Forests, were selected for evaluation. Stock for both plantations were 2 + 1 white pine, which were outplanted in early May 1990. Both sites had been broadcast burned prior to planting. Each plantation was within the Flat Creek Drainage and consisted of approximately 18 acres on north-northeast facing exposures (figure 1).

After transplants had been in the plantation for one growing season (figure 2), they were evaluated for root colonization by potential pathogenic fungi. Ten randomly-selected transplants were carefully excavated from each plantation and transported to the laboratory for analysis. Height (from the groundline to the top of the apical bud on the main stem), caliper, extent of noticeable root decay, and amount of mycorrhizal development of fine roots were determined for each sampled transplant.

Root systems of transplants were thoroughly washed for several minutes under running tap water to remove adhering soil. Small pieces (2-3 cm in length) were cut from portions of each root system which were either noticeably decayed or appeared healthy. Root pieces were surface sterilized in a 10 percent bleach solution (0.525 percent aqueous sodium hypochlorite) for 1 minute, rinsed in sterile, distilled water, and placed on two types of selective agar media. One medium was selective for *Fusarium* and closely-related fungi (Komada 1975), while the other medium consisted of V-8 juice agar amended with several antibiotics being selective for *Pythium*, *Phytophthora*, and other related water mold fungi. At least 5 root pieces were assayed from decayed and nondecayed portions of each root system on each selective medium.

Plates with Komada's medium were incubated at about 24 degrees C. under diurnal cycles of cool, fluorescent light for 7-10 days; those with V-8 juice agar were incubated in the dark at about 22 degrees C. for 3 days. Fungi emerging from root pieces were transferred to potato dextrose agar for identification. *Fusarium* spp. were identified using the taxonomic scheme of Nelson and others (1983). Both carnation leaf agar (Fisher and others 1982) and another medium of low water potential which stimulates sporulation of some species (Nirenberg 1981) were utilized. *Cylindrocarpon* spp. were identified using the monograph by Booth (1966). Water mold fungi were identified to genus.

Decay was limited to less than 10 percent of the root system in most transplants; decay was evident by blackened coloration of the epidermis and and rapid loss of epidermal and cortical tissues (figure 3). Based on previous observations, mycorrhizae are generally common on bareroot seedlings grown within bareroot beds at the Coeur d'Alene Nursery. Mycorrhizal symbionts carried on stock from the nursery may or may not persist once seedlings are outplanted on forest sites. Roots of the white pine transplants examined had relatively low levels of noticeable mycorrhizal development on feeder roots (table 1 and figure 3).

Fusarium spp. were isolated from roots of 70 percent of the transplants sampled from plantation 3G and 80 percent from plantation 3H (table 2). However, only a small percentage of the sampled roots (17.0 percent and 11.0 percent from plantations 3G and 3H, respectively) were colonized with these fungi. Black roots (those with noticeable decay) were not colonized to a greater extent with *Fusarium* than apparently healthy roots from plantation 3G; slightly higher amounts were obtained from black roots on samples from plantation 3H. Three species of *Fusarium* were isolated: *F. oxysporum* Schlecht., *F. proliferatum* (Matsushima) Nirenberg, and *F. acuminatum* Ell & Ev. *Fusarium proliferatum* was recovered from more sampled roots than the other two species (table 2). Although *F. oxysporum* and *F. acuminatum* are potential pathogens to conifer seedlings (James and others 1989), it is not known if most isolates of *F. proliferatum* obtained from conifer seedling roots are pathogenic.

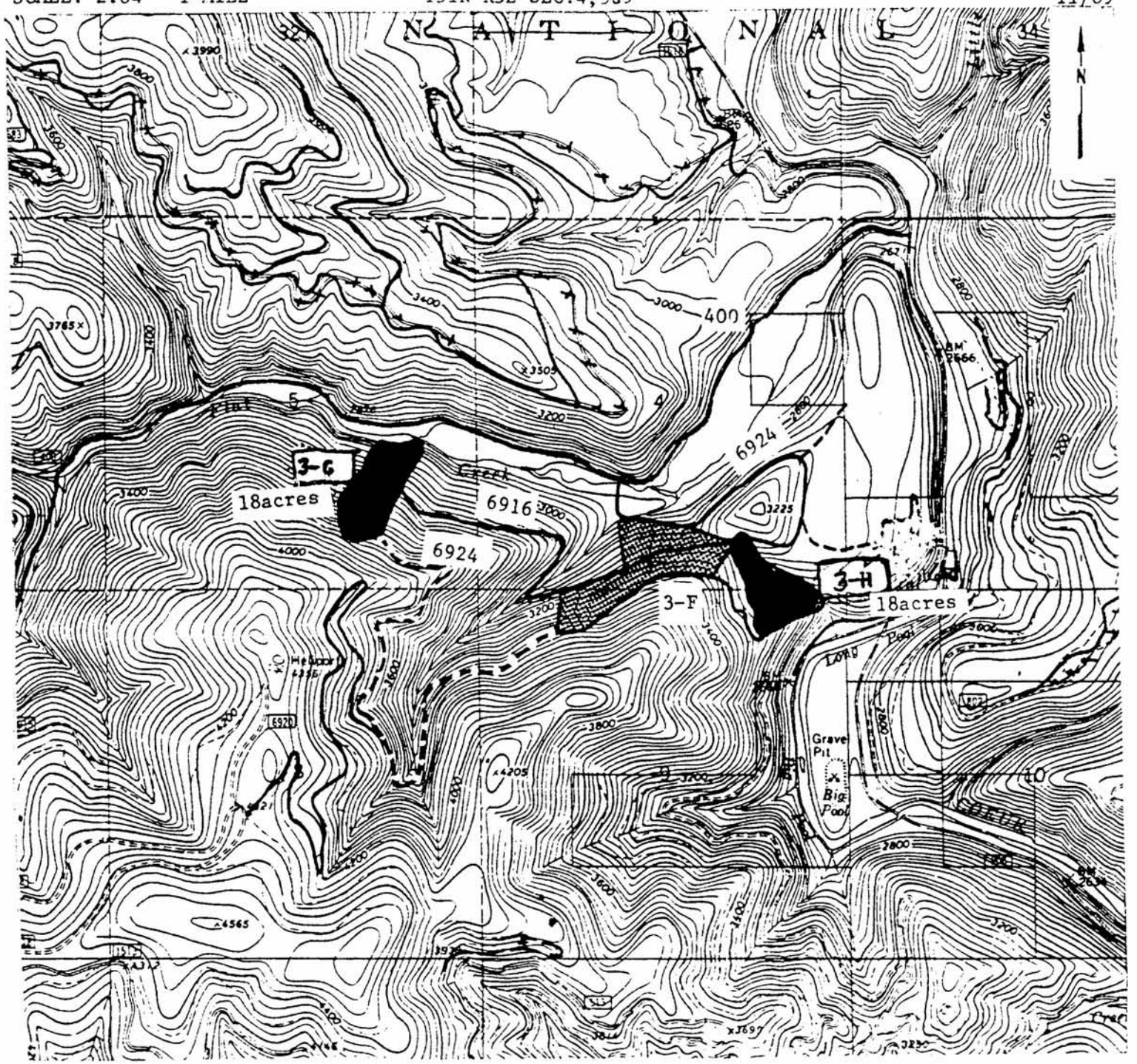

Figure 1. Location of plantations 3G and 3H within the Flat Creek drainage, Wallace Ranger District, Idaho Panhandle National Forests.

Figure 2. Western white pine 2 + 1 transplant after one growing season on the Wallace Ranger District, Idaho Panhandle National Forests.

Figure 3. Typical root system of a 2 + 1 western white pine transplant from the Wallace Ranger District, Idaho Panhandle National Forests. Arrow denotes root with decay. Note low levels of mycorrhizal development on feeder roots.

Cylindrocarpon spp. were commonly isolated from the roots of white pine transplants from both plantations (table 2). Relatively high levels of root colonization by this fungus was detected, especially on transplants from plantation 3G. *Cylindrocarpon destructans* (Zins.) Scholten was the most common species isolated; a few transplants from plantation 3G were also colonized with *C. tenue* Bugn. *Cylindrocarpon* spp. may be pathogenic or saprophytic (Booth 1966); slightly higher amounts of these fungi were detected on decayed roots in this evaluation. It is not known if these fungi might cause problems once seedlings or transplants are outplanted on forest sites.

Two of the tranplants from plantation 3H had roots which were colonized at low levels with *Pythium* spp. These fungi were only isolated from decayed roots and not found on most of the sampled transplants.

Other fungi detected on roots included *Trichoderma*, *Penicillium* and *Phoma* spp. These organisms are common saprophytes, often encountered on the roots of nursery stock. They were detected at varying levels on roots of transplants from both plantations (table 2). Levels of *Trichoderma* were especially high on transplants from plantation 3H.

None of the sampled transplants displayed above-ground symptoms of root disease similar to that commonly seen after transplanting in the nursery. Although there was some root infection by residual *Fusarium* and *Cylindrocarpon* on most transplants, these organisms were not inducing disease; at least, disease symptoms were not evident. *Fusarium* spp. on the roots of bareroot seedlings are often replaced by other mycoflora following outplanting on forest sites (Smith 1967). However, *Fusarium* spp. appear more persistent on container-grown seedlings following outplanting (Dumroese and others, unpublished), although they were not important causes of seedling mortality during the first few years on a forest site.

On the basis of this evaluation, it appears that potentially pathogenic fungi may be carried on the roots of transplants from the nursery and persist for at least one growing season. However, they do not initiate important levels of mortality soon after outplanting. Transplants on the sites sampled were healthy and did not appear stressed. Apparently, sufficient moisture was available for their successful establishment and first-year growth. It is possible that residual potential pathogenic fungi are more damaging on seedlings which may be stressed following outplanting.

Table 1. Size and root characteristics of western white pine transplants sampled for potentially pathogenic fungi from the Wallace Ranger District, Idaho Panhandle National Forests.

PLANTATION 3G

Seedling No.	Seedling Height	Size (mm) Caliper	Root Decay Rating[1]	Mycorrhizal Rating[2]
1	262	10	1	1
2	352	11	2	1
3	354	11	1	1
4	233	9	1	1
5	413	9	2	1
6	376	12	2	2
7	239	9	1	2
8	255	9	2	1
9	243	6	1	2
10	184	6	3	0
Average	291.1	9.2	1.6	1.2

[1] Based on percent of examined roots with noticeable decay (blackened roots with epidermal and cortical tissues easily removed).
- 1 = decay rare; less than 5% of the root system decayed.
- 2 = decay more common; from 5-10% of the root system decayed.
- 3 = decay common; greater than 10% of the root system decayed.

[2] Based on percent of root tips with ectomycorrhizal branching and fungal mantles.
- 0 = none
- 1 = 0-10% of root tips mycorrhizal
- 2 = 10-25% of root tips mycorrhizal
- 3 = greater than 25% of root tips mycorrhizal

Table 1, continued

PLANTATION 3H

Seedling No.	Seedling Height	Size (mm) Caliper	Root Decay Rating[1]	Mycorrhizal Rating[2]
1	302	10	1	1
2	297	11	1	1
3	300	12	1	1
4	401	12	1	2
5	325	9	1	1
6	330	10	1	2
7	250	9	1	3
8	190	8	2	2
9	174	7	1	1
10	150	5	1	2
Average	271.9	9.3	1.1	1.6

[1] Based on percent of examined roots with noticeable decay (blackened roots with epidermal and cortical tissues easily removed).
- 1 = decay rare; less than 5% of the root system decayed.
- 2 = decay more common; from 5-10% of the root system decayed.
- 3 = decay common; greater than 10% of the root system decayed.

[2] Based on percent of root tips with ectomycorrhizal branching and fungal mantles.
- 0 = none
- 1 = 0-10% of root tips mycorrhizal
- 2 = 10-25% of root tips mycorrhizal
- 3 = greater than 25% of root tips mycorrhizal

Table 2. Fungal colonization of roots of outplanted western white pine transplants from the Wallace Ranger District, Idaho Panhandle National Forests.

PLANTATION 3G

	Percent Seedlings Infected			Percent Root Colonization[1]		
	Black[2]	Brown[3]	All	Black[2]	Brown[3]	All
Fusarium						
F. oxysporum	20	30	40	3.8	5.7	4.7
F. proliferatum	20	30	30	9.4	11.3	10.4
F. acuminatum	0	20	20	0	3.8	1.8
All *Fusarium*	40	70	70	13.2	20.8	17.0
Cylindrocarpon						
C. destructans	100	80	100	69.8	56.6	63.2
C. tenue	0	20	20	0	7.5	3.8
All *Cylindrocarpon*	100	100	100	69.8	64.1	67.0
Trichoderma spp.	70	70	90	24.5	20.7	22.6
Penicillium spp.	10	20	30	1.9	7.5	4.7
Phoma spp.	60	70	80	26.4	20.7	23.6

[1] Based on percent colonization of root pieces sampled (minimum of five per seedling) by appropriate fungi.

[2] Roots with blackened epidermal and cortical tissues which were decayed and easily removed.

[3] Roots apparently healthy (no noticeable decay) with tan to brown epidermal tissues.

Table 2, continued

PLANTATION 3H

	Percent Seedlings Infected			Percent Root Colonization[1]		
	Black[2]	Brown[3]	All	Black[2]	Brown[3]	All
Fusarium						
F. oxysporum	0	10	10	0	2.0	1.0
F. proliferatum	30	30	50	10.0	6.0	8.0
F. acuminatum	10	10	20	2.0	2.0	2.0
All *Fusarium*	40	50	80	12.0	10.0	11.0
Cylindrocarpon						
C. destructans	70	30	70	24.0	8.0	16.0
Trichoderma spp.	100	100	100	76.0	82.0	79.0
Penicillium spp.	10	0	10	2.0	0	1.0
Phoma spp.	50	70	80	12.0	20.0	17.0

1 Based on percent colonization of root pieces sampled (minimum of five per seedling) by appropriate fungi.

2 Roots with blackened epidermal and cortical tissues which were decayed and easily removed.

3 Roots apparently healthy (no noticeable decay) with tan to brown epidermal tissues.

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