An Assessment of *Cylindrocarpon* on Container Western White Pine Seedlings After Outplanting

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ABSTRACT: *Cylindrocarpon root disease can destroy root systems of container seedlings. Foresters were concerned that infected nursery stock would perform poorly after outplanting. Root growth potential tests were a poor predictor of disease levels on seedlings that otherwise met standard morphological criteria (height, stem diameter, and firm root plugs) for outplanting.* Cylindrocarpon *on western white pine persists longer on outplanted stock than* Fusarium oxysporum *or* F. proliferatum *on Douglas-fir seedlings on a similar site. Mortality caused by herbivores was as serious a threat to plantation establishment as was mortality caused by all other factors. Our results suggest that western white pine seedlings infected with* Cylindrocarpon, *but meeting morphological criteria for outplanting, will perform satisfactorily in the field. West. J. Appl. For. 15(1):5–7.*

Fungi in the genus *Cylindrocarpon* are found in both forest (Thornton 1960) and nursery environments (Beyer-Ericson et al. 1991), and are common rhizosphere inhabitants of container-grown conifer nursery stock (James et al. 1994, Kope et al. 1996). *Cylindrocarpon* spp. are readily isolated from roots of diseased and apparently nondiseased seedlings in the inland Pacific Northwest, and although *C. tenue*, *C. didymum*, *C. cylindroides*, and *C. destructans*, are found, *C. destructans* is the most common species (James et al. 1994). Often, infected plants fail to show aboveground symptoms (i.e., chlorotic or necrotic foliage), but may have extensive root decay. Western white pine (*Pinus monticola*) appears especially susceptible to Cylindrocarpon root disease (James and Gilligan 1990, James 1992, James et al. 1994).

A few studies have examined the effects of outplanting stock to forest soils when the stock is infected with pathogens found in nurseries. Smith (1967) found levels of *Fusarium oxysporum* on roots of outplanted, bareroot sugar pine (*Pinus lambertiana*) seedlings decreased over time and after 4 yr could not be detected. Douglas-fir (*Pseudotsuga menziesii*) seedlings with low levels of *Fusarium* infection in their roots appeared to grow and survive as well as noninfected seedlings on a forest site, and after 4 yr *Fusarium* could no longer be detected on seedling root systems (Dumroese et al. 1993). Survival of outplanted bareroot Douglas-fir seedlings infected with *Phytophthora* spp. was directly related to disease severity of seedlings in the nursery; although *Phytophthora* persisted on outplanted seedlings, the fungus failed to colonized new roots (Hansen et al. 1980). Bareroot black spruce (*Picea mariana*) seedlings infected with *Cylindrocladium floridanum* and showing symptomatic shoots or primary roots had lower survival than seedlings with symptomatic lateral roots or nonsymptomatic shoots (Saunders et al. 1992).

In December 1992, during operational extraction of a seedlot of western white pine (Moscow Arboretum 1989) from a Montana nursery, many container seedlings with nonsymptomatic, vigorous-looking shoots lacked sufficient roots to maintain plug integrity. Often, these seedlings had only a tap root because of extensive decay of fibrous higher order roots. Root assays revealed infection by *Cylindrocarpon* spp., mostly *C. destructans*. An assay from a subsample of the entire crop, including both acceptable and cull seedlings, indicated about 50% of the crop was infected and average root colonization of the sampled population was 63%. Average seedling height was 12.5 cm, average root collar diameter (RCD) was 3.0 mm, and the frost hardiness LT_{50} was –24°C.

Using seedlings that made contract specifications for height, stem diameter, and root plug integrity, our objectives were to (1) determine if a standard root growth potential test

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Table 1. Site characteristics.

Site	Owner	Location	Elevation (m)	Aspect	Slope (%)
Titley Creek	Potlatch Corporation	Lat. 47°01'08"N Long. 116°11'50"W	980	Southwest	15
Trestle High Bank	Potlatch Corporation	Lat. 47°03'03"N Long. 116°14'14"W	1,025	East	30
Tyson Peak	Idaho Department of Lands	Lat. 47°05'02"N Long. 116°27'15"W	1,140	Southeast	40

could assist in predicting potential outplanting success of infected seedlings, (2) monitor seedling survival and growth on forest sites, and (3) determine the persistence of the pathogen after outplanting.

Methods and Materials

Seedlings were operationally packed during December 1992 and freezer stored (–2°C) until March. From March until testing or planting, seedlings were kept in refrigerated storage near 3°C.

Preplanting Root Growth Potential (RGP) Test

During March 1993, 75 seedlings meeting contract specifications were randomly collected from storage boxes and planted into 4 liter pots (5 seedlings/pot) filled with 1:1 peat moss:vermiculite. Seedlings were grown 3 wk in a greenhouse under natural photoperiod with temperatures ranging from 15 to 27°C. Seedlings were carefully harvested and the roots gently washed with running tap water. New roots were counted by category: < 1 cm and ≥ 1 cm. From each seedling, 10 root pieces, each 1 cm long, were randomly selected and removed from the original plug. If available, 10 more root pieces from new roots were also removed, and all pieces placed onto Komada's (1975) medium. Root pieces were incubated 7 days under cool fluorescent light at ≈24°C. Samples were assayed for infection; percentage root colonization was expressed as the number of root pieces infected within the entire sample of root pieces.

Outplanting Sites

In April 1993, 300 seedlings were planted on each of 3 sites in northern Idaho, near Clarkia, about 65 km northeast of Moscow. Each site (Table 1) was harvested during 1992, broadcast-burned during fall 1992, and classified as a *Thuja plicata/Clintonia uniflora* habitat type (Cooper et al. 1991). At outplanting, 15 soil samples taken to a depth of 10 cm were randomly taken from each site and assayed for *Cylindrocarpon* using the dilution plate method of Nash and Snyder (1962). During each fall from 1993 through 1997, a random sample of 20 seedlings from each site was excavated, measured for height, and roots assayed for *Cylindrocarpon* as described in the RGP test. In 1997, seedling root collar diameter was also measured.

Statistical Analysis

For the RGP experiment, mean *Cylindrocarpon* infection on new roots and plug roots was compared with a paired t-test. Correlations between new root growth and *Cylindrocarpon* infection and colonization were examined with unweighted least squares linear regression. We compared mean infection and colonization levels of *Cylindrocarpon* on seedling roots over time using a one-way analysis of variance. Tukey's HSD was used to separate means when the P value was ≤ 0.05.

Results and Discussion

During the RGP experiment, all seedlings grew new roots.The average number of new short roots (< 1 cm) ranged from 0 to 82, with a median of 26. The average number of new long roots (≥ 1 cm) ranged from 0 to 82 with a median of 23. After the test, root assays indicated 71% of the seedlings were infected by *C. destructans*. There was no difference in infection between plug roots and new roots (P = 0.8). Colonization intensity of plug roots was 28%, while new roots were less colonized at 14% (P = 0.0002). New short roots (SR), new long roots (LR), and total roots produced were unaffected by *C. destructans* infection ($P \geq 0.8$). SR and LR numbers were unaffected by increasing levels of *C. destructans* colonization on plug roots (SR $P = 0.4$; LR $P = 0.6$) or new roots (SR $P = 0.9$; LR $P = 0.2$). Attempts to predict *C. destructans* intensity on new roots from either SR or LR numbers were unsuccessful; r^2 values ≤ 0.05. Our ability to predict *C. destructans* intensity on plug roots from SR or LR numbers was also low; r^2 values ≤ 0.12.

Root growth on nearly 75% of the crop was class 4 or higher (11 to 30 new roots longer than 1 cm) on Burdett's (1979) RGP scale (5 is maximum). Low levels of root growth (Burdett's class 2 or less), which may adversely impact outplanting success, occurred on about 12% of the seedlings. Interestingly, of the seedlings with very low levels of root growth, 70% were infected with *Cylindrocarpon*, but infected seedlings had significantly (P = 0.03) more new roots than the 30% without *Cylindrocarpon*.

After outplanting, seedling mortality averaged about 6% each year for the first three growing seasons (Table 2). Mortality due to herbivory was less a factor during the first growing season than death due to physiological dysfunction (dead but not eaten), but after 5 yr, death due to herbivores or physiological dysfunction was similar (Table 2).

Table 2. Mean height (± standard error), survival, and cumulative mortality by type for all sites combined.

Year	Height (cm)	Survival	Cumulative mortality	
			Herbivores	Other
		(%)		
1993	22 ± 1	93	2	5
1994	33 ± 1	87	5	8
1995	48 ± 2	81	9	10
1996	64 ± 2	78	11	11
1997	82 ± 2	76	12	12

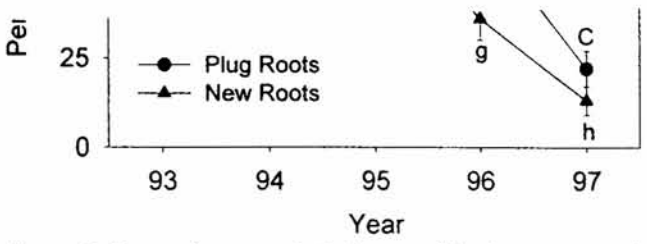

Figure 1. For each year and all sites combined, percentage (± standard error) of seedlings with original plug roots and/or new roots infected with *Cylindrocarpon*. Different letters for each root type indicate significantly different means at $P = 0.05$.

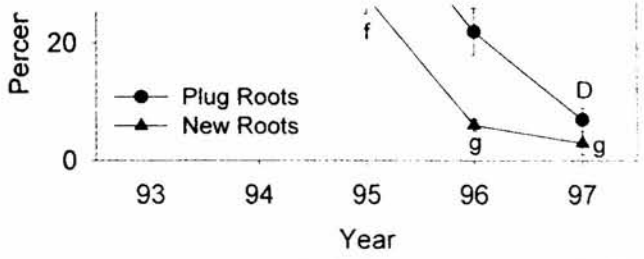

Figure 2. For each year and all sites combined, colonization percentage (± standard error) of original plug roots and new roots by *Cylindrocarpon*. Different letters for each root type indicate significantly different means at $P = 0.05$.

Unlike an earlier study with *Fusarium oxysporum* on bareroot Douglas-fir seedlings (Smith 1967) and another study with *F. oxysporum* and *F. proliferatum* on container Douglas-fir on a northern Idaho site (Lat. 47°02'N, Long. 116°01'W) with similar elevation, habitat type, slope, and aspect (Dumroese et al. 1993), *Cylindrocarpon* spp. were still isolated from white pine seedlings 5 yr after outplanting. However, both the number of seedlings infected and colonization intensities decreased over time (Figures 1 and 2). On infected seedlings, new roots generally were infected less often and had lower colonization intensities than original plug roots (Figures 1 and 2). Only 25% of the noneaten dead seedlings found after 1995 were infected with *Cylindrocarpon*. Some infection from soil-borne *Cylindrocarpon* populations may have occurred, as *C. tenue* was isolated from Titley Creek soil samples at very low levels [14 colony forming units (CFUs) per gram of soil compared to 1900 CFUs for *Trichoderma* spp., an ubiquitous potential antagonist] and *C. destructans* was found at Trestle High Bank at very low levels compared to *Trichoderma* spp. (24 CFUs to 1375 CFUs). However, even though *Cylindrocarpon* was found in Titley Creek soil, it was the only site where seedling assays failed to detect the pathogen during the final year of the study.

Seedlings grew well after planting, averaging 14 cm of new growth each year (Table 2). We detected no differences in height growth between infected and noninfected seedlings (data not shown).

Management Implications

Fungi in the genus *Cylindrocarpon*, particularly *C. destructans*, can cause serious damage to seedling crops in container nurseries. From our examination of infected western white pine seedlings in the nursery and after outplanting, it appears that seedling crops meeting morphological criteria for outplanting will perform satisfactorily in the field.

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