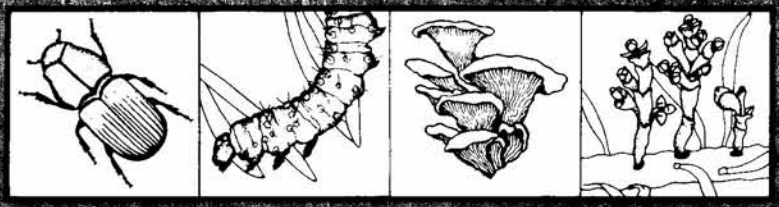
Forest Pest Management



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CYLINDROCARPON ROOT DISEASE OF CONTAINER-GROWN WHITEBARK PINE SEEDLINGS USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Extensive mortality of container-grown whitebark pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho was due to root disease caused by *Cylindrocarpon destructans*. This fungus was detected at high levels on roots of both healthy and diseased seedlings; it was also consistently isolated from damped-off germinants and containers in which diseased seedlings grew. *Fusarium* spp., especially *F. oxysporum*, were commonly isolated from whitebark pine seedcoats but were less often found colonizing roots of diseased seedlings. An emphasis on sanitation of containers and interiors of greenhouses, and increased monitoring of seedling crops for presence of potential pathogens to dictate chemical fungicide applications, will help control this disease in the future.

INTRODUCTION

There is currently extensive demand for whitebark pine (*Pinus albicaulis* Engelm.) seedlings for planting on national forests of the Northern Region for grizzly bear habitat. Growers at the USDA Forest Service Nursery in Coeur d'Alene, Idaho have recently begun producing whitebark pine seedlings in plastic "super cell" containers to meet this demand for wildlife habitat. Whitebark pine seeds are large, often dormant for long time periods, and require scarification for germination.

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[1] Stationed in Coeur d'Alene, Idaho.

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During spring 1989, a crop of whitebark pine seedlings were sown in containers at the nursery. Because of very slow growth, two growing seasons were required to produce seedlings of suitable outplanting size. At the end of the first growing season, most seedlings appeared healthy. However, in the spring and early summer of the second growing season, seedling tops began to turn necrotic (figure 1). Affected seedlings were scattered more or less randomly throughout container trays (figure 2). In some affected seedlings, necrosis was concentrated on the new (current season) growth; many affected needles died back from their tips (figure 1). Examination of affected needles failed to reveal presence of foliage pathogens. Necrotic foliage was often twisted, a symptom sometimes associated with wilting and root disease of 5-needle pines (James 1987a, 1990). Growers were quite concerned about the rapid appearance of dying seedlings despite applications of pesticides. Initial samples of affected seedlings were analyzed by PENISU-LAB (a private diagnostic lab in Kingston, WA). The five seedlings from this sample yielded low levels of *Phytophthora* and *Fusarium* and much higher levels of *Cylindrocarpon* on roots. Fungal species were not identified.

MATERIALS AND METHODS

During July 1990, an evaluation was conducted to determine quantitative association of fungi on seed, damped-off germinants, and roots of older whitebark pine seedlings. Comparisons were made of fungi colonizing roots of seedlings with and without disease symptoms. Root systems of sampled seedlings were washed thoroughly under running tap water for a few minutes to remove growing media. Pieces (2-3 cm in length) were excised from root systems, surface sterilized for 1 min in a 10 percent bleach solution (0.525 percent aqueous sodium hypochlorite), rinsed in sterile water and placed on selective media. Two selective media were used: one is selective for *Fusarium* spp. and closely related fungi (Komada 1975), and the other, selective for *Pythium* and associated "water mold" fungi, is composed of V-8 juice agar amended with several antibiotics including pimaricin, rifamycin, and ampicillin. Plates of Komada's media were incubated under diurnal cycles of cool, fluorescent light for 7-10 days; those with V-8 juice agar were incubated in the dark for 3 days. Selected fungi growing from incubated root pieces were transferred to potato dextrose agar (PDA) for identification. The major monographs used for fungal species identification were those of Booth (1966), Middleton (1943), and Nelson and others (1983).

Seedcoats from randomly collected whitebark pine seed were assayed for major fungal colonizers. Selected seed were aseptically dissected with the outer and inner seedcoat placed directly on Komada's medium. Ten "super cell" containers, in which diseased seedlings grew, were assayed by extracting four small pieces from the bottom with a sterile scalpel; pieces were placed on Komada's medium. A new crop of whitebark pine seedlings (sown in spring 1990) were examined for post emergence damping-off. Several seedlings displaying typical damping-off symptoms (hypocotyl attacked at the groundline and bent over) were collected, washed, surface sterilized and plated on Komada's medium. All plates with Komada's medium were incubated as described above. Selected fungi emerging from seedcoats, pieces of container, and damped-off seedlings were identified.

In a related investigation, 50 seedlings with intermediate or advanced disease symptoms were removed from their containers, their roots washed thoroughly under running water for several minutes to remove adhering particles of media, and carefully transplanted in sterilized peat-vermiculite growing media within sterilized plastic containers. The seedlings were monitored for 9 months; those which died were sampled for potentially pathogenic fungi on their roots. At the end of 9 months, all surviving seedlings were removed from their containers and sampled for colonization by root fungi.

Figure 1.--Whitebark pine container-grown seedling with foliar chlorosis and necrosis indicative of root disease.

Figure 2.--Distribution of diseased whitebark pine seedlings in a tray of "super cell" containers from the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

RESULTS AND DISCUSSION

Root isolations from whitebark pine seedlings with and without root disease symptoms are summarized in table 1; those from damped-off seedlings, seedcoats, and "super cell" containers are summarized in table 2. Root disease development in transplanted seedlings and isolations from their roots are summarized in table 3. The most common fungus consistently isolated from diseased and non-diseased seedlings, damped-off seedlings and containers was *Cylindrocarpon destructans* (Zins.) Scholten. Another *Cylindrocarpon* species, *C. tenue* Bugn., was isolated infrequently from some seedlings. *Fusarium* spp. were isolated from about half of the diseased and non-diseased seedlings, but root colonization rates were much lower than with *Cylindrocarpon* spp. The four *Fusarium* spp. isolated from roots included *F. acuminatum* Ell. & Ev., *F. proliferatum* (Matsushima) Nirenberg, *F. oxysporum* Schlecht. and *F. solani* (Mart.) Appel & Wollenw. *Pythium ultimum* Trow. was also isolated from a few seedlings, but colonization rates were quite low. Other fungi frequently isolated from seedlings, seedcoats, and containers included common saprophytes, such as *Trichoderma* and *Penicillium* spp. Although infrequently colonizing roots of whitebark pine seedlings, *Phoma* spp. were routinely isolated at high levels from containers, where they are common residents (James and others 1988).

Consistent high association of *Cylindrocarpon destructans* with seedling roots indicated that this fungus was responsible for the pathology of whitebark pine seedlings. This fungus was isolated at very high levels from roots of diseased as well as healthy seedlings, bottoms of containers, and recently damped-off seedlings. However, the fungus was an uncommon colonizer of whitebark pine seed. On the other hand, most sampled seedcoats were colonized with *Fusarium* spp., especially *F. oxysporum*. These common seed-borne fungi (Dumroese and others 1988) can potentially initiate seedling disease. The fact that they were not recovered at high levels from roots of diseased seedlings indicates their minor role in disease etiology.

Cylindrocarpon destructans has a long history of involvement with conifer seedling diseases (Booth 1966; James 1988). The fungus is a common cause of pre- and post-emergence damping-off (Vaartaja and Crum 1956), root disease of older seedlings (James 1987b; James and Gilligan 1990), and is a common rhizosphere colonizer of conifer seedlings (Kluge 1966; Kowalski 1980). It is an opportunist which becomes pathogenic under conducive environmental conditions (Matturi and Stenton 1964a). Production of toxic metabolites which initiates host cell necrosis sometimes occurs despite physical absence of the fungus in diseased tissues (Evans and others 1967; Wilhelm 1959). Like some species of *Fusarium*, *C. destructans* can be either an aggressive pathogen or saprophytic (Booth 1966; James 1988). Environmental conditions like moisture extremes and nutritional characteristics of the host play major roles in pathogenicity (Dennis and Sutherland 1989; Ross 1960; Rouatt and others 1963). Likewise, different genetic strains of the fungus probably exist, some of which are more pathogenic than others (Unestam and Bayer-Ericson 1990). In any event, consistent association of *C. destructans* with roots of diseased whitebark pine seedlings in this investigation probably indicates that it was an aggressive pathogen.

This investigation re-confirms that *Cylindrocarpon* spp. are capable of colonizing surfaces of plastic containers used to grow seedlings (James and others 1988; James and Gilligan 1988). The fungus produces chlamydospores which can remain viable in a dormant state for a long time (Booth 1966; Domsch and others 1980). These resting spores are activated when a susceptible host is nearby (Matturi and Stenton 1964b); they are likely the form in which the fungus is carried from one crop to another within contaminated containers. Apparently, seed contamination was not an important source of inoculum for these seedlings. Other possible inoculum sources might be the interior of greenhouses including benches, floors, and walls. Based on previous experience (James 1985), it is expected that the peat-vermiculite growing medium used for these seedlings was not extensively contaminated with *Cylindrocarpon*.

Table 1.--Colonization of whitebark pine seedling roots by selected fungi
USDA Forest Service Nursery, Coeur d'ALene, Idaho.

Seedlings with Root Disease Symptoms[1]

	Percent Colonization		
Fungus	Seedlings	Intact Root Pieces[2]	Detached Root Pieces[3]
Cylindrocarpon			
destructans	83.9	61.1	52.7
tenue	22.6	12.6	1.8
All *Cylindrocarpon*	97.0	71.8	54.5
Fusarium			
proliferatum	32.2	6.0	16.4
oxysporum	9.7	3.8	0
acuminatum	22.6	7.9	0
solani	3.2	2.7	0
All *Fusarium*	58.1	20.0	16.4
Pythium ultimum	30.8	4.8	NA[4]
Trichoderma spp.	41.9	7.4	47.3
Penicillium spp.	9.7	0.8	5.4
Phoma spp.	45.2	18.6	3.6

Seedlings without Root Disease Symptoms[5]

Cylindrocarpon			
destructans	100.0	80.0	83.3
tenue	25.0	3.3	0
All *Cylindrocarpon*	100.0	83.3	83.3
Fusarium			
proliferatum	25.0	1.7	4.8
acuminatum	25.0	1.7	0
All *Fusarium*	50.0	3.3	4.8
Pythium ultimum	25.0	5.0	NS[4]
Trichoderma spp.	75.0	13.3	28.6
Penicillium spp.	0	0	0
Phoma spp.	25.0	15.0	2.4

[1]Thirty-one seedlings with root disease symptoms sampled on Komada's medium and 26 seedlings sampled on V-8 juice agar.
[2]Roots still attached to seedlings after they were extracted from containers. From 10-15 root pieces sampled per seedling; most sampled root pieces from near the tips of lateral roots.
[3]Roots detached from seedlings during extraction from containers. Five randomly collected root pieces sampled per seedling.
[4]Not sampled on V-8 juice agar (for *Pythium* spp.).
[5]Four seedlings without root disease symptoms sampled on both Komada's medium and V-8 juice agar. Sample was insufficient for statistical comparisons with diseased seedlings.

Table 2.--Colonization of whitebark pine damped-off seedlings, seedcoats, and "super cell" containers from the USDA Forest Service Nursery, Coeur d'Alene, Idaho, with selected fungi.

	Percent Colonization[1]		
Fungus	Seedlings	Seedcoats	Containers[2]
Cylindrocarpon			
destructans	54.4	61.1	52.7
tenue	22.6	12.6	1.8
All *Cylindrocarpon*	97.0	71.8	54.5
Fusarium			
proliferatum	32.2	6.0	16.4
oxysporum	9.7	3.8	0
acuminatum	22.6	7.9	0
solani	3.2	2.7	0
All *Fusarium*	58.1	20.0	16.4
Pythium ultimum	30.8	4.8	NA[4]
Trichoderma spp.	41.9	7.4	47.3
Penicillium spp.	9.7	0.8	5.4
Phoma spp.	45.2	18.6	3.6

[1]Sample sizes: damped-off seedlings = 11; seedcoats = 38; containers = 10.

[2]First number is percent of sampled cells colonized and number in parentheses is percent of sampled pieces (four per cell) colonized.

Table 3.--Root disease development and fungal root colonization of transplanted whitebark pine seedlings with disease symptoms from the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Percent seedling Survival[1] Month						
July	August	September	October	November	March	Apr
	---------- 1990 ----------				--- 1991 ---	
100	72	24	20	18	6	4

	Percent Seedling Colonization[2]	
Fungus	Dead Seedlings	Live Seedlings
Cylindrocarpon destructans	100	100
Fusarium		
proliferatum	48	0
oxysporum	6	50
acuminatum	15	0
All *Fusarium*	60	50
Trichoderma spp.	81	100
Penicillium spp.	13	0
Phoma spp.	42	0

[1]Fifty seedlings with intermediate to severe root disease symptoms transplanted into sterile peat-vermiculite media in July 1990. Seedlings monitored at about monthly intervals until April 1991.

[2]Percentage of seedlings sampled which were colonized with appropriate fungi. "Dead seedlings" died during the experiment; live seedlings survived until April 1991.

DISEASE MANAGEMENT

To prevent severe losses from this disease in the future, care should be taken to provide and maintain a clean greenhouse environment, use new or sterilzed containers, and periodically remove diseased individual seedlings. If damping-off becomes apparent early in the crop, treatment with fungicides such as benomyl or captan is recommended. Periodic diagnosis of associated root-pathogenic organisms is important to initiate proper control approaches. For example, preliminary analysis of diseased whitebark pine samples indicated a possible role of *Phytophthora* with the disease. However, further investigations failed to confirm presence of these important pathogenic fungi on diseased seedlings. Therefore, fungicide treatments should emphasize chemicals like benomyl and captan which have greater efficacy against *Cylindrocarpon* and *Fusarium* rather than those designed to control "water mold" fungi. Surface sterilizing seed prior to sowing is also recommended to help reduce levels of potentially pathogenic fungi, especially *Fusarium* spp. (Dumroese and others 1988). Hot water treatments can greatly reduce levels of contaminating fungi on containers, including *Cylindrocarpon* spp. (James and Woollen 1989). It is important that either new containers or those that have been effectively sterilized be used to grow disease-susceptible crops such as whitebark pine seedlings. Recent experience has shown that high-quality crops of container-grown conifer seedlings may be produced without using high levels of chemical fungicides (Dumroese and others 1990). An integrated pest management approach with greater emphasis on sanitation may be as effective in controlling disease and potentially less detrimental to the environment as dependence on chemical treatments.

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