CHLOROSIS OF CONTAINER-GROWN WESTERN WHITE PINE SEEDLINGS FISHER RIVER RANGER DISTRICT, KOOTENAI NATIONAL FOREST, MONTANA

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Western white pine (*Pinus monticola* Dougl.) container-grown seedlings, originally planned for fall 1988 planting, but had to be stored during the winter of 1988-89 at the Fisher River Ranger District, Kootenai National Forest because they could not be planted due to excessively dry forest conditions. During the storage period, many of the seedlings developed extensive chlorosis over much of their crowns and several displayed needle-tip dieback with needles which were twisted. These symptoms were indicative of possible infection by root pathogenic fungi (James 1985, 1987a, 1987b). Therefore, analysis of association of fungal organisms with decline symptoms were conducted.

Seven seedlings displaying various levels of needle tip necrosis, foliage chlorosis, and root decay (table 1) were analyzed in the laboratory. Necrotic needle tissues were washed thoroughly under running tap water and aseptically placed in moist chambers containing filter paper saturated with sterile distilled water. Moist chambers were incubated at about 24°C for 5 days, after which they were examined for superficial growth of fungi on needle tissues. Roots of each seedling were washed thoroughly under running tap water for several minutes to remove adhering soil particles. Ten tips (2-3 mm each) were cut from randomly selected lateral roots. Tips were surface sterilized in a 10 percent aqueous bleach solution (0.525 percent sodium hypochlorite) for 1 minute, rinsed with sterile distilled water, blotted dry and aseptically placed on a selective medium for root pathogens (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light for 5-7 days after which they were examined for fungal growth from root tips. Selected fungi were transferred to potato dextrose agar slants and identified using standard taxonomic guides (Barnett and Hunter 1972; Nelson, Toussoun and Marasas 1983).

Table 1. Descriptions of foliage and root symptoms of container-grown western white pine seedlings from the Fisher River Ranger District, Kootenai National Forest.

Seedling Number	Description of Foliage and Root Symptoms
1	Needle tip necrosis prominent, at least half way down most needles. Remainder of foliage chlorotic. Tips of new foliage also necrotic. Root decay moderate with some sloughing of epidermal tissues.
2	Slight needle tip necrosis; accompanying needle banding with black fungal sporulation within the bands. Needle twisting prominent. Chlorosis of foliage general throughout crown. No root decay evident.
3	No needle tip necrosis; foliage chlorosis general for entire seedling. No root decay evident.
4	Needle tip necrosis prominent, about ¼ to ⅓ of the length of most needles. Some needle banding also evident with black fungal sporulation within bands. Chlorosis general throughout crown. Slight root decay evident.
5	Needle tip necrosis not evident, but tips are slightly violet in color. Chlorosis general throughout crown. No root decay evident.
6	Needle tip necrosis not evident. Chlorosis general throughout crown and some banding evident, although no fungal sporulation evident within bands. No root decay evident.
7	Needle tip necrosis prominent, about ½ to ⅔ of the length of most needles. Banding prominent on most needles with extensive fungal sporulation within bands. Moderate root decay evident.

Incubation of necrotic foliage in moist chambers yielded several different groups of fungi. *Lophodermium nitens* Darker was consistently sporulating within necrotic bands on needles. This fungus produced elongated, subcuticular hysterothecia within necrotic areas, and is fairly common on needles of western white pine, where it is considered a weak parasite (Darker 1932). Previous investigations (James 1983) indicated that *L. nitens* may be associated with needle-tip dieback on ponderosa pine (*Pinus ponderosa* Laws.) seedlings in nurseries. Other fungi growing superficially on the surface of necrotic needles included *Alternaria*, *Phoma*, *Penicillium*, and *Botrytis cinerea* Fr. ex Pers. This latter fungus commonly colonizes necrotic foliage, particularly of container-grown seedlings (James 1984), and may cause molding of stock during cold storage (Sutherland, Shrimpton and Sturrock 1989). However, none of these foliage fungi were present in sufficient numbers to significantly contribute to the decline of white pine seedlings.

Root isolations (table 2) yielded several different groups of fungi. Common saprophytic genera included *Trichoderma*, *Aspergillus*, and *Mucor*. *Fusarium oxysporum* Schlect. and *F. acuminatum* Ell. & Ev., both potential root pathogens of conifer seedlings (James, Dumroese and Wenny 1988), were isolated from several seedlings. However, the most consistently isolated group of fungi were *Cylindrocarpon* spp. They were isolated from 6 of 7 seedlings and more than 70 percent of the root tips sampled. Some *Cylindrocarpon* spp. are capable of causing serious diseases of conifer seedlings (James 1988); these fungi have been found associated with root deterioration of container-grown western white pine seedlings, but affected seedlings often lacked disease symptoms (James 1987c). As with most other groups of potentially pathogenic fungi, many *Cylindrocarpon* isolates are saprophytic (Booth 1966). However, under conditions of host stress, such as may have occurred during the handling and storage of these white pine seedlings, *Cylindrocarpon* spp. may have contributed to disease which was manifested by needle-tip necrosis and foliar chlorosis (James 1988). Isolation results indicated that those seedlings with evidence of root decay (table 1) were colonized to a greater extent with *Cylindrocarpon* than seedlings without root decay. It is possible that resident populations of *Cylindrocarpon* present on roots of seedlings proliferated during storage and elicited disease symptoms on seedlings which were most extensively colonized. Pathogenicity tests to clarify potential of isolates of these fungi to cause disease of western white pine seedlings would be very beneficial.

From this evaluation, it is concluded that needle-tip necrosis and foliar chlorosis of container-grown western white pine seedlings was probably the result of abnormal stress inflicted on seedlings during handling and/or storage from the time they left the nursery. It is likely that root infection by *Cylindrocarpon* spp. (perhaps aided by *Fusarium* spp.) also contributed to or accelerated seedling decline. If seedlings could have been planted during the fall of 1988 as originally scheduled, these problems would probably not have occurred. It is likely that these root fungi probably would not have caused disease or mortality of vigorous outplanted seedlings.

Table 2.--Fungal colonization of western white pine root tips - Fisher River Ranger District, Kootenai National Forest.

	Colonization Percentage	
Fungus	Seedlings	Root tips
Fusarium oxysporum	57.1	32.9
Fusarium acuminatum	28.6	8.6
Cylindrocarpon spp.	85.7	71.4
Trichoderma spp.	57.1	7.1
Aspergillus sp.	14.3	1.4
Mucor sp.	14.3	2.9

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