NURSERY DISEASE NOTES USDA FOREST SERVICE

NORTHERN REGION
FOREST HEALTH PROTECTION

No. 150 April 2003

FUNGAL ASSOCIATES OF CORKY ROOT SYNDROME ON BARE ROOT 2-0 WESTERN WHITE PINE SEEDLINGS USDA FOREST SERVICE NURSERY COEUR D'ALENE, IDAHO

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ABSTRACT

Several bare root western white pine seedlings that were evaluated for effects of different pre-plant soil treatments on production had abnormal root systems after being grown for 2 years. Above-ground portions of affected seedlings appeared healthy, but they had extensive callus production on taproots and a paucity of lateral roots. Seedlings grown in plots fumigated with dazomet had the highest percentage of these "corky roots". Isolations from abnormal root systems yield high levels of *Cylindrocarpon destructans* and *Fusarium oxysporum*. This abnormality resembles the "corky root disease" of Douglas-fir seedlings previously described in Canadian bare root nurseries. Bare fallowing instead of soil fumigation may help reduce incidence of this syndrome.

INTRODUCTION

Recent evaluations of bare root conifer seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho have emphasized developing alternatives to pre-plant soil fumigation (James et al. 1994b, 1996; Stone et al. 1997). At the end of most tests, two-year-old seedlings are routinely removed for morphological measurements of seedling biomass. Biomass comparisons among the different treatments reflect possible non-lethal effects of root pathogens (James et al. 1994b).

At the conclusion of a recent test, many western white pine (*Pinus monticola* Dougl.) seedlings that appeared healthy, i.e., lacked above-ground disease symptoms, had pronounced aberrations on their root systems (figure 1). Affected seedlings had abnormal callus growth along the taproot, usually commencing just below the soil line. There were also very few lateral roots on affected seedlings. These symptoms were similar to the "corky root disease" syndrome previously described on bare root Douglas-fir seedlings in British Columbia (Bloomberg and Sutherland 1971; Sutherland and Sluggett 1973, 1975). Corky root disease is characterized by damage from both nematodes (*Xiphenema bakeri* Williams) (Sutherland 1970, 1974; Sutherland and Dunn 1970; Sutherland and Sluggett 1974) and the soilborne fungus *Cylindrocarpon destructans* (Zins.) Scholten (Bloomberg and Sutherland 1971).

To determine possible roles of potentially-pathogenic fungi in this corky root syndrome on white pine seedlings, a series of isolations were made from symptomatic 2-0 seedlings from the Coeur d'Alene Nursery.

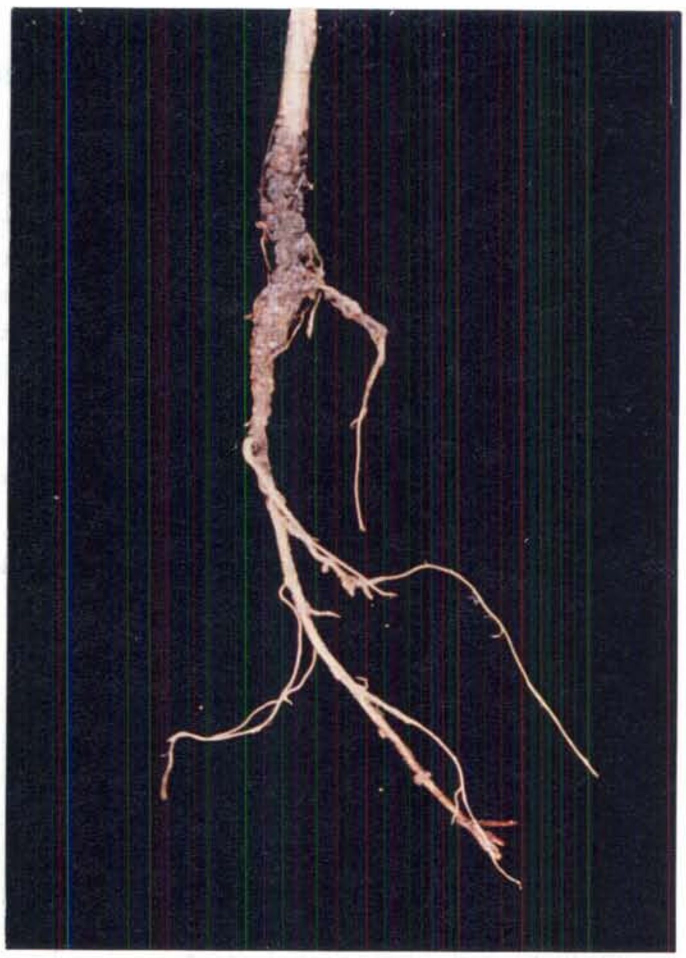

Figure 1. Bare root 2-0 western white pine seedling with corky taproot syndrome. Note abnormal callus production on taproot and paucity of lateral roots.

MATERIALS AND METHODS

Twenty-five 2-0 seedlings were randomly selected from plots used to evaluate different pre-plant soil treatments. Seven pre-sowing soil treatments had been evaluated: standard dazomet fumigation, bare fallow with monthly cultivation, steam treatment, bare fallow with biological control (*Trichoderma harzianum* Rifai - Bio-Trek) amendment, bare fallow with biological control (*T. harzianum* - University of Idaho), winter rape (*Brassica napus* L.) green manure crop incorporation with biological control (*T. harzianum* – University of Idaho) amendment, and winter mustard (*Brassica juncea* L.) green manure crop incorporation with biological control (*T. harzianum* – University of Idaho) amendment. All treatments were sown with the same white pine seedlot.

Seedlings were carefully extracted from field plots to ensure that most of their root systems remained attached. Roots were washed thoroughly to remove adhering soil particles and examined for presence or absence of corky taproots. Percent of sampled seedlings from each of the seven treatments with some level of the corky taproot syndrome was determined.

Samples of corky taproot tissues from all treatments were collated for analysis of fungal associates. Sections about 2-3 mm in length were aseptically cut through corky taproots, surface sterilized in 10% bleach (0.525% aqueous sodium hypochlorite), rinsed in sterile water, blotted dry, and placed on a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Plates were incubated at about 24°C under diurnal fluorescent light for 7-10 days. Selected emerging fungi were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar for identification using the taxonomy of Booth (1966) and Nelson et al. (1983). Percentage of sampled taproot sections colonized by selected fungi was calculated.

RESULTS AND DISCUSSION

Many more seedlings with corky taproot syndrome were grown in dazomet-fumigated soil (table 1). Other treatments resulted in much lower levels of affected seedlings. The lowest levels of seedlings with corky taproot syndrome were grown in steam treated soil or in soil with an incorporated winter rape green manure crop amended with the biological control agent (table 1).

Several potentially-pathogenic fungi were isolated from corky taproot tissues (table 2). The most common were *Fusarium* spp., primarily *F. oxysporum* Schlecht., and *Cylindrocarpon destructans*. *Fusarium solani* (Mart.) Appel & Wollenw. was also isolated, although at much lower levels. *Trichoderma* spp., which are common nursery soil inhabitants (James 1989; James and Beall 1999, 2000; Papavizas 1985) and the biological control agent added in four of the seven pre-plant soil treatments (table 1), were isolated at extremely low levels. This would seem to indicate that *Fusarium* and *Cylindrocarpon* colonization of root tissues was not much inhibited by *Trichoderma* populations.

Corky root disease was first described in 1963 on Douglas-fir seedlings in British Columbia (Bloomberg and Sutherland 1971). This disease was characterized by swollen tap roots, a paucity of lateral roots, and stunted shoot growth. The first two symptoms were common on the 2-0 white pine seedlings from the Coeur d'Alene Nursery described in the current evaluation (figure 1). The disease on Douglas-fir in Canada was associated with damage by the nematode *Xiphinema bakeri* followed by extensive colonization primarily by *C. destructans* (Sutherland and Sluggett 1973, 1974, 1975). However, *C. destructans* was generally isolated at higher levels during the winter, whereas both *Fusarium oxysporum* and another common soil fungus (*Mycelium radicis atrovirens*) were more commonly isolated during the summer (Bloomberg and Sutherland 1971). In the current evaluation, seedlings were sampled in the fall at the end of the second growing season; these seedlings were colonized at nearly equal levels by both *C. destructans* and *F. oxysporum*. The selective medium used in this evaluation would probably not allow detection of *Mycelium radicis atrovirens* and extractions of associated nematodes were not undertaken. Therefore, possible roles of these other organisms in the disease syndrome on white pine seedlings are unknown.

Because of similarity of root symptoms and colonization of corky taproot tissues by *C. destructans* and *F. oxysporum*, it is suspected that this abnormality is very similar to the "corky root disease" described for Douglas-fir bare root seedlings in Canada. It is possible that undetected nematodes may have been associated with abnormal taproot morphology on white pine seedlings. Occasional distortion of tap- and lateral roots on container-grown whitebark pine seedlings, similar to those seen in the bare root white pine, have previously been observed (James 2000; James et al. 1994a). These abnormalities have commonly been associated with high levels of root colonization by both *C. destructans* (James 1991, 2000) and *Fusarium* spp. (James 2000).

It is interesting that the highest level of corky taproot-affected seedlings were found in plots fumigated with dazomet. This fumigant is usually quite effective in killing soilborne pathogens, including *F. oxysporum* and *C. destructans* (James 1989; James and Beall 1999; James et al. 1996) as well as other potential pathogens such as nematodes (Barnard et al. 1994; Boone 1988; Hildebrand and Dinkel 1988; James 1989). It is possible that pathogenic fungi and perhaps nematodes capable of causing the corky root disease were reintroduced into fumigated plots on machinery or from adjacent, non-fumigated soils. Another possibility is that these pathogens reinvaded fumigated soil from below the effective zone of fumigation (James 1989; Vaartaja 1967). Fumigated plots were not amended with biocontrol agents and were essentially "biological vacuums" after treatment (Boone 1988; James 1989). Therefore, organisms invading fumigated soil would have very few other organisms to compete with and might quickly reach high population levels (James 1989; Vaartaja 1967). In any event, there were sufficient populations of both *C. destructans* and

F. oxysporum, and perhaps soil nematodes as well, to result in high levels of root colonization and inducement of the corky root syndrome.

Workers in Canada found that bare fallowing fields with periodic cultivation, particularly during the warm summer months, greatly reduced population levels of fungal and nematode associates of corky root disease (Bloomberg and Sutherland 1971; Sutherland and Sluggett 1975). Low levels of corky taproot syndrome on white pine seedlings were found in fallowed plots, including those amended with biocontrol agents (table 1).

Although this disease did not result in noticeable seedling mortality, stunting or chlorosis, affected seedlings would be culled because of their abnormal root systems, especially the lack of good lateral root development. Therefore, this problem would result in significant losses.

Table 1. Pre-sowing soil treatment effects on percent of bare root 2-0 western white pine seedlings with corky taproot syndrome - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Pre-plant Soil Treatment	Percent Affected Seedlings
Dazomet fumigation	64
Bare fallow with cultivation	20
Steam treatment	15
Bare fallow with BioTrek®	28
Bare fallow with UI Biocontrol	28
Winter rape with UI Biocontrol	16
Winter mustard with UI Biocontrol	36
Average of all treatments	29.6

Table 2. Colonization of corky taproot tissues from bare root 2-0 western white pine seedlings by selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fungus	Percent Colonization
Fusarium oxysporum	65.3
Fusarium solani	15.3
All *Fusarium* spp.	78.0
Cylindrocarpon destructans	68.7
Trichoderma spp.	1.3

MANAGEMENT IMPLICATIONS

This disease syndrome requires further investigation, especially concerning possible roles of nematodes and potential susceptibility of other conifer species. If nematodes are found to be involved, soil treatments that reduce populations should be investigated. Although losses currently are at fairly low levels, bare root seedling crops should be periodically monitored for extent of this problem.

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