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CYLINDROCARPON DESTRUCTANS ASSOCIATED WITH ROOT DISEASE OF CONTAINER-GROWN WESTERN WHITE PINE TREE IMPROVEMENT STOCK USDA FOREST SERVICE NURSERY COEUR D'ALENE, IDAHO

R.L. James

ABSTRACT

Cylindrocarpon destructans was isolated from nearly all sampled root pieces from three western white pine trees exhibiting dieback/wilting systems while being grown in large containers for tree improvement purposes at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Other potentially-pathogenic fungi were isolated at insignificant levels. It is likely that *C. destructans* was the major cause of tree decline. Three distinct morphotypes of this fungus were isolated from white pine root pieces. Decline symptoms were probably exacerbated by maintaining wet root systems of affected trees for extended periods. Fungicide drenches may help limit disease severity, but effective control can only be obtained by maintaining adequate drainage, avoiding over-watering, and ensuring trees are grown under fairly acidic conditions. Young five-needle pine trees are very susceptible to root decay by *Cylindrocarpon*, especially when roots exposed to high moisture conditions for prolonged periods.

INTRODUCTION

Western white pine (*Pinus monticola* Dougl.) is an important species in the tree improvement program at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. A extensive program of screening for resistance to white pine blister rust (*Cronartium ribicola* Fisch.) is ongoing. Part of this program involves growing some progeny with high resistance potential for prolonged periods in large [gallon] containers. These trees are grown in standard growing media, usually comprised of peat moss mixed

with either sawdust, perlite or vermiculite.

During the early summer of 2003, several of these white pine container-grown trees began showing decline or wilting symptoms (figure 1). Dieback was evident from the tips of branches; needles lost chlorophyll and generally remained light in color rather than turning dark brown or red (figure 2). Some needles also became twisted, which is characteristic of wilting in five-needle pines (James 1984, 1987a, 2000; James et al. 1994).

Growers wanted to know the cause of tree decline. Therefore, an evaluation was conducted to determine extent and identity of organisms capable of eliciting disease.

MATERIALS AND METHODS

Three trees exhibiting decline symptoms were sampled for root pathogens. Trees were removed from containers and roots with obvious decay symptoms were cut from root systems. Sampled roots were mostly attached to trees, although a few detached roots were also sampled. Roots were washed thoroughly to remove adhering particles of growing media. Fine roots (< 3 mm in diameter) were selected from collected larger roots for isolations. Roots from all three trees were collated, cut into pieces about 5 mm in length, surfaced sterilized with 10% bleach (0.525% aqueous sodium hypochlorite), rinsed in sterile water, blotted dry, and aseptically placed on two types of selective agar media. Fifty root pieces (5 per plate-10 plates) were incubated on a medium selective for Oomycete organisms (*Pythium; Phytophthora*). This medium consisted of V-8 juice agar amended with the antibiotics pimaricin, rifamycin, and ampicillin and the fungicide pentachloronitrobenzene (James et al. 1990, 1996a; Stone et al. 1995). Plates with V-8 juice agar were incubated in the dark at about 24°C for 3 days. *Pythium* colonies were identified on the basis of their diameter after 3 days (15-20 mm), feathery margin, and growth within rather than superficially on the agar surface. No *Phytophthora* was isolated. There were 153 remaining root pieces; these were incubated on a selective medium for *Fusarium* and closely-related species (Komada 1975). Plates of Komada's medium were incubated under diurnal cycles of cool, fluorescent light at about 24°C for at least 7 days. Emerging fungi were transferred to potato dextrose agar and carnation leaf agar (Fisher et al. 1982) and identified using the taxonomy of Nelson et al. 1983 and Booth 1966. *Pythium* isolates were identified using the procedures and taxonomy of Waterhouse (1968). Percentages of root pieces colonized by different fungi were calculated.

Figure 1. Western white pine tree with terminal leader decline symptoms USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Figure 2. Dieback symptoms on declining western white pine trees grown in large containers at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Note bleaching of chlorophyll and twisting of needles.

RESULTS

Most sampled root pieces from declining white pine trees were colonized by *Cylindrocarpon destructans* (Zins.) Scholten. (table 1). Isolations from colonized root pieces yielded three distinct morphotypes of this fungus (figure 3). Major differences among the morphotypes included pigmentation and extent of aerial mycelium produced in culture. All morphotypes produced macro- and microconidia as well as chlamydospores characteristic of *C. destructans* (Booth 1966). The largest percentage of *C. destructans* isolates obtained from white pine roots was placed in the third morphotype (table 1). This group had fairly abundant aerial mycelium and dark tan to brown pigmentation (figure 3). The other morphotypes were isolated less frequently; morphotype 1 had a pionnotal appearance with masses of macroconidia produced in sporodochia, but lower numbers of microconidia and chlamydospores. Morphotype 2 generally produced fewer spores than either of the other two morphotypes.

Pythium irregulare Buisman was isolated from only 2 of the 50 sampled root pieces (4%). *Fusarium proliferatum* (Matsushima) Nirenberg was likewise isolated from only 2 of the sampled root pieces (1.3%)(table 1). Other isolated fungi included the saprophytes *Trichoderma, Penicillium, and Phoma* spp., all of which were isolated infrequently.

DISCUSSION

Cylindrocarpon destructans is commonly associated with diseased container-grown five-needle pine species in the Northern Region (James 1984, 1987b, 1987c, 1990, 1992; James and Gilligan 1990). This fungus has routinely been isolated from diseased western white or whitebark pine seedlings at the Coeur d'Alene Nursery, often associated with high levels of root decay (James 1985a, 1985b, 1987a, 1991a, 1991b, 1991c, 1995, 1998, 2000, 2003; James and Gilligan 1986; James et al. 1996b). Severely-infected seedlings may not necessarily display disease symptoms. However, once seedlings are extracted from containers, extensive root decay is often evident (James 1988; James et al. 1994, 1996b). The consistent association of *C. destructans* with decay symptoms and its reported pathogenicity on conifer seedlings (Beyer-Ericson et al. 1991; Bonello and Pearce 1993; Bonello et al. 1991; Buscot et al. 1992; Dahm and Strezelczyk 1987), implicates this fungus in disease etiology. Losses to particular crops at some nurseries have been extensive (James 1987c, 1990, 1992).

Cylindrocarpon destructans is a common rhizosphere colonizer that can elicit disease on plant roots without necessarily colonizing them (Buscot et al. 1992; Holdenreider and Sieber 1992). The fungus produces toxins that kill host cortical cells (Beyer-Ericson et al. 1991; Buscot et al. 1992); these toxins are probably produced prior to actual invasion of host root cells (Buscot et al. 1992). Once the fungus has successfully invaded plant roots, it initiates decay by producing a wide range of enzymes that break down cell walls (Beyer-Ericson et al. 1991; Dahm and Strzelczyk 1987).

Table 1. Colonization of roots from declining tree improvement western white pine trees by selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Root Pieces[1]	*Cylindrocarpon destructans*[2]				*Fusarium* spp.[3]	Other Fungi[4]
	Morphotype 1	Morphotype 2	Morphotype 3	All Morphotypes		
Number[5]	24	53	78	147	2	16
Percent	15.7	34.6	51.0	96.1	1.3	10.5

[1] Total of 153 root pieces from three sampled trees
[2] Morphotype differences based on colony morphology on PDA
[3] All isolates *F. proliferatum*
[4] Includes *Trichoderma, Penicillium,* and *Phoma* spp.
[5] Number colonized by particular fungus.

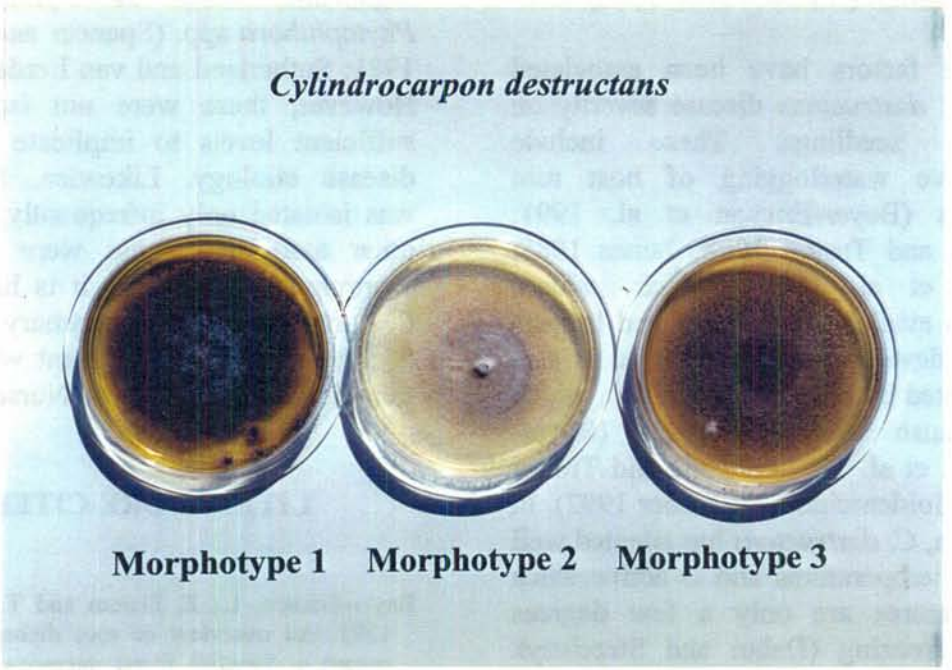

Figure 3. Morphotypes of *Cylindrocarpon destructans* isolated from roots of declining container-grown western white pine trees - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Several different strains of *C. destructans* may be associated with diseased host plants (Dahm and Strzelczyk 1987; Holdenreider and Sieber 1992). Some of these strains are undoubtedly more virulent than others, but whether level of virulence is related to easily-identifiable characteristics such as cultural morphology is unknown. Three rather distinct morphotypes of *C. destructans* were isolated from roots in this investigation. They were isolated at differing frequencies, with one morpho-

type being more common than the others. This morphotype has frequently been associated with root decay of five-needle pines (James 2000, 2003; James et al. 1996b), and may be the most prevalent strain associated with conifer seedling roots. Studies using genetic markers would help elucidate the variability within *C. destructans* populations and perhaps could be used to identify the most virulent pathogenic strains. Recently, PCR (polymerase chain reaction) techniques were developed to diagnose *C. destructans* on diseased conifer seedlings, without the need for isolation, culturing and fungal identification (Hamelin et al. 1996).

Several factors have been associated with *C. destructans* disease severity on conifer seedlings. These include excessive waterlogging of host root systems (Beyer-Ericson et al. 1991; Dennis and Trotter 1998; James 1988; James et al. 1994), which reduces oxygen availability to roots and hastens fungal development. The fungus is also stimulated by alkaline conditions, which also cause stress on conifers (Beyer-Ericson et al. 1991; Dennis and Trotter 1998; Holdenreider and Sieber 1992). In addition, *C. destructans* has adapted well to low temperatures and is active when temperatures are only a few degrees above freezing (Dahm and Strzelczyk 1987; Dennis and Trotter 1998).

Because *C. destructans* has relatively low inherent virulence, i.e., it is generally considered a weak pathogen (Bonello and Pearce 1993; Bonello et al. 1991; Dennis and Trotter 1998), environmental conditions and/or host stresses are important influences on disease occurrence (James 1988; James et al. 1994). Therefore, disease severity can be reduced by maintaining plant vigor by ensuring adequate drainage in containers, avoiding over-watering, and keeping root system pH below about 5.5 (Beyer-Ericson et al. 1991; Dennis and Trotter 1998; Holdenreider and Sieber 1992). When disease symptoms become evident, root system decay is usually extensive and drenching plant roots with fungicides has little effect (Beyer-Ericson et al. 1991; James 1988; James et al. 1994).

Dieback and decline of large plants grown in containers may sometimes be caused by infection with Oomycete organisms, such as *Pythium* and *Phytophthora* spp. (Spencer and Benson 1981; Sutherland and van Eerden 1980). However, these were not isolated at sufficient levels to implicate them in disease etiology. Likewise, *Fusarium* was isolated only infrequently, and the other associated fungi were probably saprophytes. Therefore, it is likely that *C. destructans* was the primary cause of decline of tree improvement white pine trees at the Coeur d'Alene Nursery.

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R.L. James is Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection. Address: USDA Forest Service, 3815 Schreiber Way, Coeur d'Alene, ID 83814; email rjames@fs.fed.us.