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**PHYTOPHTHORA BLIGHT OF
CONTAINER-GROWN *CEANOTHUS* SEEDLINGS –
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ABSTRACT

Native *Ceanothus integerrimus* seedlings grown in containers at the USDA Forest Service Nursery, Coeur d'Alene, Idaho were recently affected by a disease resulting in root, stem and leaf necrosis. The most noticeable symptoms on affected seedlings were blackened main stems, some of which appeared bent over similar to "shepherd's crooks". Necrosis initially started in root systems and then spread through the main stem into seedling crowns. Eventually, entire seedlings were killed or rendered too damaged for shipment. Isolations from diseased seedling root and stem tissues consistently yielded *Phytophthora cinnamomi*. Other *Phytophthora* species have infrequently been found at the Coeur d'Alene Nursery; this is the first report of *P. cinnamomi* at the nursery. This potentially-damaging pathogen is usually restricted to warm and wet habitats. Its potential as a seedling pathogen may be restricted to container operations in north Idaho. Procedures for reducing damage caused by this important pathogen are discussed.

INTRODUCTION

Seedling production at the USDA Forest Service Nursery, Coeur d'Alene, Idaho has historically emphasized reforestation conifers for the Northern Region. However, in recent years, several new plant species have been grown at the

nursery to provide stock for a wide range of uses, including restoration of native plants. Many native plant species have only recently been produced at the nursery. Therefore, problems discovered during their production have not often been encountered before. A disease problem was recently discovered on container-grown deer brush (*Ceanothus*

integerrimus Hook. & Arnold) seedlings at the nursery. This disease resulted in necrotic seedling tops and appeared during the final stages of the growth cycle when seedling canopies were dense. The disease started within the roots and spread into main stems and seedling crowns. In severely-affected seedlings, all above-ground tissues became necrotic. Root systems exhibited a dry-type of decay. Although mortality was not extensive, several groups of seedlings were affected. Since this disease was unfamiliar, investigations were conducted to determine presence and identity of associated potentially-pathogenic organisms in order to develop management strategies that might reduce future damage.

MATERIALS AND METHODS

Seedlings from several diseased groups were selected for laboratory analysis. Selected seedlings, obtained from the edges of disease groups, displayed some necrosis, but contained sufficient healthy tissues to indicate active pathogenesis. Seedlings were washed thoroughly and portions of necrotic stems and branches were incubated in moist chambers to promote sporulation of associated fungi. Isolations were also made from the margins of necrotic stems, i.e., where necrotic tissues were adjacent to healthy tissues. Small pieces of margin tissue were surface sterilized in 0.525% aqueous sodium hypochlorite, rinsed in sterile, distilled water and incubated on 2% water agar (WA) for 24 hrs. Emerging fungi were transferred to potato dextrose agar. After microscopic examination, selected isolates were grown for a few days in the dark on V-8

juice agar amended with pimarin, rifamycin, ampicillin, and pentachloronitrobenzene; this medium is selective for water mold fungi, primarily those in the genera *Pythium* and *Phytophthora* (James 2000; James et al. 1991). Water mold fungi were identified using the taxonomy of Waterhouse (1956, 1963, 1968) and Middleton (1943). Species identification was facilitated by growing test isolates on slants of 2% WA for a few days and then flooding the plates with non-sterile pond water to induce sporulation.

Isolations were also made from the root systems of several diseased seedlings. Root were washed thoroughly to remove adhering growing media, surface sterilized and rinsed as described above and incubated directly on V-8 juice agar. Emerging fungi were transferred to new V-8 juice agar plates and incubated for 5 days at 24°C in the dark. Associated water mold fungi were identified using pond water to stimulate sporulation as described above.

To determine presence of potentially-pathogenic water mold fungi within the container growing medium, apple baits were used as traps. Media from several diseased seedlings was placed in solution within a large beaker in sterile, distilled water. Fresh apples were suspended in the solution for 24 hrs., removed and incubated at about 24°C in the dark for an additional 48 hrs. Apple tissue from selected necrotic lesions was aseptically transferred to V-8 juice agar plates and incubated as described above. Selected water mold isolates were identified.

RESULTS AND DISCUSSION

A few isolations revealed presence of *Pythium* spp., especially *P. irregulare* Buisman. and *P. ultimum* Trow. However, the most consistently-isolated, potentially-pathogenic fungus was identified as *Phytophthora cinnamomi* Rands. This fungus was routinely isolated from decayed feeder roots and the margins of necrotic stem lesions; it was also commonly obtained from peat-vermiculite growing media of diseased seedlings.

Phytophthora spp. have infrequently caused diseases in bareroot production areas at the Coeur d'Alene Nursery but has never previously been found in container operations. *Phytophthora cactorum* was previously isolated from basal lesions of western larch tree improvement stock (James 1993) and *P. cactorum* and *P. megasperma* were associated with root diseased bareroot western larch seedlings (James 2000). Most water mold-associated diseases at the nursery have been caused by various *Pythium* spp.; these diseases are usually located within bareroot seedling production areas where water drainage is impaired, resulting in prolonged water-saturated soils (James 1982; James et al. 1991).

Fungal isolates obtained from diseased container-grown *Ceanothus* seedlings were identified as *P. cinnamomi* based on hyphal characteristics as well as morphology of sporangia and chlamydospores (Ann and Ko 1985; Ho et al. 1983; Waterhouse 1963). This species has a world-wide distribution on

many different plant species (Ribeiro 1989), but is most often restricted to warmer/tropical rather than temperate environments (Ann and Ko 1985; Bega 1974; Benson 1982). Nursery seedlings, including conifers (Benson 1984; Campbell and Verrall 1963; Cooley et al. 1985; Hamm and Hansen 1982) may be attacked by the pathogen; such diseases have usually been restricted to bareroot production areas (Atkinson 1982; Cooley et al. 1985). However, the pathogen may infect container-grown ornamental stock (Benson 1990; Hardy and Sivasithamparam 1991a; Hoitink et al. 1977) where damage seems often related to poor water drainage of growing media (Benson 1986; Bumbieris 1981; Davison and Tay 1987).

Phytophthora cinnamomi usually resides in infested soil as resting structures called chlamydospores (Benson 1987; Grassia et al. 1984; Hwang and Ko 1978; Kliejunas and Ko 1976; Reynolds et al. 1985). Under suitable environmental conditions when susceptible hosts are present, chlamydospores germinate and either cause root infection directly or develop sporangia which produce motile zoospores which can initiate infection (Ann and Ko 1985; Aveling and Rijkenberg 1989; Hyde et al. 1991). Zoospores and hyphae from chlamydospores are attracted to host roots because of complex carbohydrates exuded by these roots (Aveling and Rijkenberg 1989). Exudates occur at the highest levels and root infection is greatest on young, developing feeder roots (Aveling and Rijkenberg 1989; Hwang and Ko 1978; Marx 1970). Older, lignified roots are not nearly as susceptible to infection (Cahill et al.

1989; Marks and Smith 1985). Zoospores encyst on root surfaces, produce infection pegs which directly penetrate root epidermal cells, thus initiating infection. Once within roots, the fungus causes decay, rendering infected roots incapable of nutrient and moisture uptake. As the disease progresses, affected plants begin to decline (Cahill et al. 1989; Dawson and Weste 1982; Hyde et al. 1991); the fungus may spread from infected roots into the main stem and, if the humidity is high enough, into the top portion of seedlings (Kenerley and Bruck 1983; Marks and Smith 1985; Ribeiro 1989).

Phytophthora cinnamomi is a unique *Phytophthora* species in several ways. The fungus produces very distinctive hyphal swellings and sporangial proliferation when grown in water culture (Ho et al. 1983, 1984; Waterhouse 1956, 1963). The sexual stage (oospores) is only formed when both A1 and A2 mating types are present (Ann and Ko 1985; Huberli et al. 1997; Kliejunas et al. 1977; Linde et al. 1997; Old et al. 1984). Therefore, the fungus is heterothallic. In many locations, only one mating type is present, so the sexual stage, and associated genetic recombination does not occur (Kliejunas and Ko 1976; Kliejunas et al. 1997; Linde et al. 1997; Shepherd et al. 1974). Oospores are common resting structures by which the pathogen withstands prolonged periods without host infection (Ann and Ko 1985; Huberli et al. 1997; Shepherd and Pratt 1974; Waterhouse 1956). When oospores are not present, the fungus survives via chlamydospores (Benson 1987; Grassia et al. 1984).

Recent molecular and genetic analyses have indicated that *P. cinnamomi* occurs

as a wide range of diverse strains (Coffey et al. 1984; Gabor et al. 1993; Linde et al. 1997). Populations vary with regards to pathogenicity on different hosts (Shepherd et al. 1974), isozyme production (Linde et al. 1997; Old et al. 1984; Oudemans and Coffey 1991), tolerance to antibiotics (Leary et al. 1982), growth rate (Zentmyer et al. 1976), and persistence in soil (Ann and Ko 1985; Halsall 1982). It is likely that the pathogen originated in Asia (particularly New Guinea or Indonesia) and was introduced into the Americas on soil or plant material transported by European explorers (Zentmyer 1977, 1988).

The pathogen probably spreads from one infected seedling to another either as zoospores in water or via mycelial growth when seedlings physically touch one another (Atkinson 1965; Averling and Rijkenberg 1989). Under warm, wet conditions, the fungus grows rapidly and can develop extensive amounts of inoculum capable of rapidly spreading the disease (Halsall and Williams 1984). Natural spread of the fungus in containers may be limited by physical separation of seedlings, reduced moisture (lower levels of irrigation or improved drainage of plugs) and cool temperatures (Benson 1982; Blaker and MacDonald 1981, 1983; Davison and Tay 1987; Halsall and Williams 1984). Growing seedlings in a medium that provides adequate water drainage and proper spacing of seedlings in containers should help improve disease control.

Fungicides can usually provide some level of disease control. If applied as soil drenches when disease is first noticed, fungicides may greatly restrict pathogen spread and subsequent disease.

However, if severe disease is common, fungicides are usually only partly effective (Bruck and Kenerley 1983; Hoitink and Schmitthenner 1975). Efficacious fungicides include metalaxyl (Subdue®) and fosetyl-Al (Alliette®); both of these have specific activity against water mold fungi, but not other pathogens commonly found in nurseries (Atkinson 1982; Benson 1984, 1987, 1990; Coffey and Joseph 1985). It is important that these fungicides be used sparingly and at proper label rates in order not to place too much selection pressure on pathogenic isolates to develop resistance (Atkinson 1982; Benson and Grand 2000).

Phytophthora cinnamomi can be effectively managed in nurseries by biological control agents, including fungal species such as *Trichoderma* (Chambers and Scott 1995), *Gliocladium* (Chambers and Scott 1995), *Micromonospora* (El-Tarabily et al. 1996), and *Myrothecium* (Gees and Coffey 1989). Biocontrol agents are especially efficacious when incorporated into growing media prior to sowing container crops (Hardy and Sivasithamparan 1991b; Hoitink et al. 1977; Marks and Smith 1985).

Hot water has been effective in disease therapeutics by treating seedlings either prior to transplanting in nurseries (45°C for 15 min – Benson 1978) or outplanting in the field (48-50°C for 5-15 min.- Theron et al. 1982).

It is possible that infection of *Ceanothus* seedlings by *P. cinnamomi* was an isolated occurrence and may not commonly occur in the future. This pathogen is greatly restricted by cold temperatures, although resting structures

can survive freezing temperatures for short time periods (Kenerley and Bruck 1983). The pathogen has been detected in coastal nurseries in Oregon and warmer portions of North Carolina and the Southeast (Benson and Grand 2000; Bruck and Kenerley 1983; Hamm and Hansen 1982; Hendrix and Campbell 1968; Mistretta 1984; Roth 1963; Roth and Kuhlman 1963). However, it has not been reported in nurseries in colder climates, probably because it does not survive the prolonged colder winter temperatures (Ho et al. 1984; Kirby and Grand 1975). Therefore, this pathogen was not expected to occur and cause disease at the Coeur d'Alene Nursery. However, it is likely that *P. cinnamomi* could only proliferate and induce disease under the warmer and wetter conditions prevailing in container operations. Hopefully, it will be unable to spread and cause significant disease problems in bareroot operations at the nursery; it is expected that the pathogen would not overwinter well outside at the nursery. However, if winters continue to become milder than in the past, this fungus may become an important pathogen at the Coeur d'Alene Nursery in the future.

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