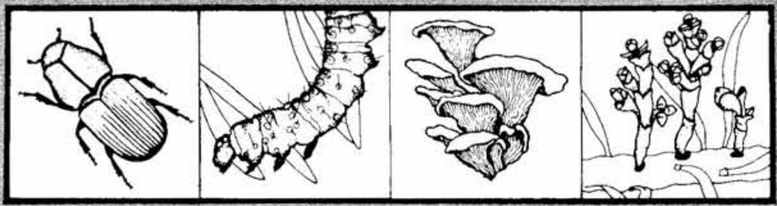
Forest Pest Management



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ROOT DISEASE OF CONTAINER-GROWN ENGELMANN SPRUCE AND WESTERN WHITE PINE SEEDLINGS PLUM CREEK NURSERY, PABLO, MONTANA

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ABSTRACT

Dwarfing and chlorosis of container-grown western white pine seedlings at the Plum Creek Nursery in Pablo, Montana was due to extensive root decay by *Cylindrocarpon destructans*. Similar symptoms on Engelmann spruce seedlings were caused by root infection with *Pythium ultimum*. Above-ground symptoms typical of container seedling root disease were not prominent on affected seedlings. Although *Cylindrocarpon*-associated root decay has occurred frequently in the past at the nursery, *Pythium* root disease is fairly rare at this and other container conifer nurseries in the northern Rocky Mountains. Sources of *Pythium* inoculum at the nursery are not known, although possibilities include contaminated seed, growing media, containers, nearby vegetation, and blowing soil. Factors affecting epidemiology of *Pythium* in container nurseries and possible approaches for reducing inoculum introductions and improving efficacy of future control efforts are discussed.

INTRODUCTION

During production of the 1991 crop of container-grown conifer seedlings at the Plum Creek Nursery (Pablo, Montana), growers located several groups of seedlings which were somewhat shorter than adjacent seedlings. Some of the shortened seedlings were also slightly chlorotic. Careful examination of the seedlings revealed that they had deteriorated root systems with abnormally high levels of decay. Decay was especially concentrated near the root tips. Affected roots often had epidermal and/or cortical tissues which were easily sloughed off, revealing stele tissue underneath (figure 1).

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Figure 1.--Lateral root of Engelmann spruce container-grown seedling which was colonized with *Pythium ultimum*, causing sloughing of epidermal and cortical tissues revealing intact stele tissues below.

Most affected seedlings were either western white pine (*Pinus monticola* Dougl.) or Engelmann spruce (*Picea engelmanni* Parry); they usually did not display typical above-ground root disease symptoms of container-grown seedlings, such as terminal or needle dieback and prominent necrotic foliage (James 1985, 1986a). However, affected seedlings could easily be located because they were shorter and slightly chlorotic when compared with surrounding seedlings.

Root disease of container-grown conifer seedlings has not been a common problem at the Plum Creek Nursery in the past even though some disease caused by *Fusarium* (James 1986b) and root decay associated with *Cylindrocarpon* spp. (James and Gilligan 1990) has been periodically detected at the nursery. Seedlings infected with *Cylindrocarpon* may not be evident until extraction from containers at the end of the growing season. Associated decay may be extensive, resulting in reduced root development. Severely-affected seedlings have been culled, sometimes resulting in reduced production levels.

MATERIALS AND METHODS

Several seedlings which were slightly shorter than normal with chlorotic foliage were evaluated for presence of fungi capable of causing root diseases. Root systems of selected seedlings were washed thoroughly under tap water to dislodge pieces of growing media. Roots were then aseptically dissected into pieces approximately 5 mm in length. Ten randomly selected root pieces from each seedling were placed on each of two selective agar media used for isolation of root-pathogenic fungi. One medium was developed by Komada (1975) for isolation of *Fusarium* and closely related fungi. The other medium contained V-8 juice agar amended with pimaricin and several other antibiotics. This medium is selective for water mold fungi such as *Pythium* and *Phytophthora* spp.

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RESULTS AND DISCUSSION

Isolations from white pine seedlings yielded *Cylindrocarpon destructans* (Zins.) Scholten on 46.7 percent of the root pieces from two-thirds of the seedlings plated on Komada's medium. *Fusarium acuminatum* Ell. & Ev. was also isolated from 6.7 percent of the root pieces (30 percent of the seedlings) incubated on this medium. Isolations from white pine seedling roots on V-8 juice agar failed to yield any water mold-type fungi. Based on previous investigations at the nursery (James 1987; James and Gilligan 1990), occurrence of *Cylindrocarpon* spp. may be common on roots of white pine seedlings; these fungi may cause extensive root decay, resulting in important losses.

Isolations from Engelmann spruce seedling roots did not yield *Cylindrocarpon* or *Fusarium* on Komada's medium. However, all sampled seedlings were extensively infected with *Pythium* when assayed on V-8 juice agar; 96 percent of the root samples yielded isolates of this fungus. Examination of the *Pythium* isolates obtained and comparisons with descriptions in taxonomic treatises (Middleton 1943; Waterhouse 1967) indicated that the isolates were *P. ultimum* Trow. Placing isolates within this taxon was based on presence of monoclinous antheridia (usually produced just below the oogonium), aplerotic oospores, and spherical sporangia usually borne at the terminal ends of hyphae (Hendrix and Papa 1974).

Although *Pythium*-associated diseases are common in bareroot conifer production (Edmonds and Heather 1973; Hendrix and Campbell 1968; James and others 1990), they are not nearly as common in container operations, at least in the northern Rocky Mountains. Therefore, little is know about the epidemiology of these fungi on container-grown conifer seedlings. The following discussion outlines information mostly from other cropping systems that may be useful in combating this disease in container nurseries.

Pythium ultimum is one of the most widespread and damaging members of the genus (Middleton 1943; Stanghellini 1974). It has an extremely wide host range and affects many different types of plants in a wide variety of habitats (Hendrix and Campbell 1973; Lifshitz and Hancock 1984; Stanghellini 1974), including conifer seedlings (Edmonds and Heather 1973; Hendrix and Campbell 1968). Although not commonly described as a pathogen of container-grown conifer seedlings, the fungus has recently been implicated as causing a needle dieback disease of young container-grown Douglas-fir germinants (Husted 1988). The species is notorious for causing decay of small feeder roots (Hendrix and Campbell 1968) and can be an aggressive pathogen under a wide range of environmental conditions (Hendrix and Campbell 1973).

Pythium ultimum often infects juvenile tissues first and then spreads to older tissues (Hendrix and Campbell 1973). The fungus usually causes most damage in wet environments (Bateman 1961; Short and Lacy 1976), even though it does not readily produce motile zoospores (Lifshitz and Hancock 1983). The wet environment (standing water, poor soil aeration) probably increases disease damage because of detrimental effects on plant hosts, rather than improved pathogenicity of *P. ultimum* (Griffin 1963; Leach 1947). High soil moisture also increases levels of host root exudates which stimulates activity of pathogens (Kerr 1964; Lifshitz and Hancock 1983; Stanghellini and Hancock 1971a). *P. ultimum* is also favored in wet soils because of its ability to tolerate low oxygen tensions (Kerr 1964). Cool temperatures (15-10°C) may also stimulate disease development by *P. ultimum* (Thomson and others 1971).

Pythium ultimum normally invades susceptible host roots quickly, causing decay; it then becomes quiescent in the form of resting structures (chlamydospores and oospores)(Hendrix and Campbell 1973; Stanghellini and Hancock 1971b). Spread of the fungus within a greenhouse environment is probably accomplished by water splash of spores resulting from overhead irrigation (Hendrix and Campbell 1973). Percolation of spores (sporangia) through growing mix columns probably is an important way the fungus moves within an individual container cell. It is not known whether resting structures of the fungus persist on containers from one seedling crop to the next like other root-pathogenic fungi (James and others 1988).

Pythium spp. may initially be introduced into a crop either in infested growing media (Robertson 1973) or in irrigation water (Hendrix and Campbell 1983; Shokes and McCarter 1979). Sphagnum peat, a common component of the growing media used to produce conifer seedlings, has been shown to be conducive to *Pythium* spp. (Robertson 1973). It is also known that *Pythium* spp. may be introduced into greenhouses via contaminated soil on machinery and/or workers and by windblown dust (Stephans and others 1983), as well as on conifer seed (Anderson 1986; James and Genz 1982). Because of its extensive host range, *P. ultimum* may also be present on other host plants located near conifer seedling greenhouses. Common weeds, grasses, and herbaceous plants may harbor this fungus, often without displaying disease symptoms (Hendrix 1974; Hendrix and Campbell 1983). *Pythium ultimum* is an extremely well adapted saprophyte on many different kinds of plant hosts (Lifshitz and Hancock 1984). It is possible that the fungus entered the conifer crop at Plum Creek Nursery from nearby plants serving as sources of initial inoculum. Infested soil moving into greenhouses from adjacent fields is another possibility, especially since the side walls of houses are raised during much of the growing season. Irrigation water used at the nursery is probably not an important source of inoculum because this water comes from deep wells where the pathogen probably does not exist. However, if pond or irrigation ditch water were used as sources of irrigation, contamination by *Pythium* would be much more likely (Shokes and McCarter 1979).

Once *Pythium* is introduced into a crop, it is very difficult to control (Stephans and others 1983). Traditional control approaches often include application of chemical fungicides when seedlings display disease symptoms. However, fungicide applications to control root diseases of container-grown conifer seedlings has not generally been successful because of problems translocating high enough pesticide concentrations to sites of infection within root zones (James 1986a). Several chemical fungicides have been developed especially for and are specific against water mold fungi. Examples include metalaxyl (Ridomil®; Subdue®), fosetyl-al (Aliette®) and ethazol (Truban®)(Pawuk 1979). These fungicides are usually applied as drenches either with portable spray equipment or in overhead irrigation systems. Efficacy problems include reduced penetration to root zones because of interception by seedling canopies and leaching chemicals through root plugs by excessive irrigation. Although *Pythium*-associated diseases are best treated when seedlings are young, before pathogens build up and spread in greenhouses, extreme care must be taken when using chemical fungicides on young plants. Juvenile seedling tissues are usually much more susceptible to toxicity by chemicals, including fungicides. Application of chemical fungicides also has the disadvantage of worker exposure problems and potential contamination of the environment, including seepage into groundwater. Because of potential problems, many growers are looking seriously into alternatives to chemical pesticides for control of diseases.

chemicals such as bleach (Dumroese and others 1988; James and Genz 1981) or hydrogen peroxide (Barnett 1976; James and Genz 1981) or subjecting seed to a continuous running water rinse for at least 48 hours (Dumroese and others 1988). These techniques are effective in eliminating most seedcoat contaminating fungi, including those in the genus *Pythium* (James and Genz 1981). Another option is steam treatment of growing media to ensure removal of root-pathogenic fungi prior to use (Baker and Olson 1959). Steam treatment, rather than complete sterilization, is preferable because such treatments do not destroy many beneficial microorganisms (such as spore-forming bacteria) which are useful in suppressing disease (Baker and Olson 1959). Pathogenic fungi, on the other hand, are more susceptible to and readily killed by steam. Another option is to incorporate organic material that promotes suppressiveness to root-pathogenic fungi into growing media, such as composted tree bark (Chen and others 1987). Growing media that is suppressive to *Pythium* contains high populations of mesophilic organisms which exhibit intense microbial activity resulting in low concentrations of available nutrients (Chen and others 1988).

Biological control of root pathogenic fungi has been investigated for a long time. However, recent efforts in this field have greatly increased, primarily because of problems encountered when relying on traditional chemical pesticides (Baker and Cook 1974). Several different fungi (Al-Hamdani and others 1983) and bacteria (Howell and others 1988; Nelson 1988) are either antagonistic toward or competitive with *Pythium* spp. The most commonly studied fungi which are capable of biologically controlling *Pythium* diseases include those in the genera *Trichoderma* (Haskins and Gardner 1978), *Gliocladium* (Howell 1982) and other species of *Pythium* (Deacon and Henry 1978; Martin and Hancock 1980). Important bacterial antagonists include several *Pseudomonas*, *Bacillus* and *Enterobacter* spp. (Baker and Cook 1974; Howell and others 1988). Some of these organisms produce antibiotics which kill or restrict growth of pathogens (Haskins and Gardner 1978; Howell 1982); others occupy niches that pathogenic *Pythium* spp. would otherwise colonize (Martin and Hancock 1980). Some biological control agents actually parasitize *Pythium* mycelium and propagules in the soil (Martin and Hancock 1980). Several commercial preparations of biological control agents are becoming available and should be tested in container seedling operations. Although those currently available have not been specifically developed for conifer seedling systems, they may prove efficaceous because they show promise against several of the pathogens that cause problems on conifer seedlings, such as *Pythium* and *Fusarium*.

Early detection of disease is important in an overall strategy to reduce disease losses. Periodic examination and confirmation of associated pathogenic fungi are necessary for susceptible species, including western white pine and Engelmann spruce. Seedlings beginning to display disease symptoms should be assayed for pathogenic fungi on their roots. If found to be present, direct control can be more effectively applied. However, preventative measures, sanitation, and incorporation of biological control agents will be at least as effective as chemical pesticides in the long run. Growers should seriously consider relying more heavily on these approaches to disease management in the future to preclude problems of environmental contamination with toxic chemicals. Alternative approaches also give growers more versatility if certain pesticides become unavailable or if their use becomes restricted by governmental regulations.

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