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Observations on the association of *Cylindrocarpon* spp. with diseases of container-grown conifer seedlings in the inland Pacific Northwest of the United States

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ABSTRACT

Cylindrocarpon spp. are common rhizosphere inhabitants of different conifer species grown in containers at nurseries in the inland Pacific Northwest of the United States. These fungi are commonly isolated from roots of diseased and apparently non-diseased seedlings. Infected plants may or may not exhibit above-ground symptoms; seedlings may have extensive root decay without indications of chlorotic foliage. *Pinus monticola* and *P. albicaulis* are especially prone to root decay by *Cylindrocarpon*. The most common species isolated from roots of diseased and non-diseased seedlings is *C. destructans*. Other isolated species include *C. tenue, C. didymum, and C. cylindroides*. Tests indicate *C. destructans* isolates are more aggressive pathogens than other species. Epidemiological studies evaluating inoculum sources, sporulation and spore distribution, and environmental factors influencing infection and symptom production are needed. Fate of *Cylindrocarpon* on roots of outplanted pine seedlings and roles of these fungi in affecting seedling performance and survival is being evaluated.

INTRODUCTION

Fungi in the genus *Cylindrocarpon* are important rhizosphere colonizers of conifer trees (Booth, 1966; Buscot *et al.*, 1992; Stenton, 1958). They are

considered pioneer colonizers of roots of many diverse plant species (Dahm and Strzelczyk, 1987a). These fungi are commonly encountered in both forest (Thornton, 1960) and nursery environments (Beyer-Ericson *et al.*, 1991; Chakravarty and Unestam, 1987). However, their possible role as pathogens of conifers has been questioned (Salt, 1979). Some reports indicate *Cylindrocarpon* spp. are important pathogens, especially under certain environmental conditions (Chakravarty *et al.*, 1991; Dahm and Strzelczyk, 1987b; Salt, 1979), whereas others indicated many are mostly saprophytes (Booth, 1966; Matturi and Stenton, 1964) or non-disease causing endophytes (Fisher and Petrini, 1990). In some cases, *Cylindrocarpon* spp. can elicit seedling disease without actually colonizing root tissues, probably because of toxin production (Chakravarty and Unestam, 1987; Evans, 1967; Unestam and Beyer-Ericson, 1990). This toxin nectrolide elicits disease symptoms similar to those caused by actual root infection (Evans, 1967). Because of their questionable role as conifer pathogens, less research has been conducted on behavior and soil interactions of *Cylindrocarpon* spp., compared to other soil-borne pathogens such as *Fusarium* spp.

We began evaluating root diseases of container-grown conifer seedlings in nurseries in the inland Pacific Northwest of the United States in the early 1980s. These evaluations were prompted by disease losses higher than most growers would tolerate. Losses of Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco), western larch (*Larix occidentalis* Nut.), and Engelmann spruce (*Picea engelmanni* Parry) seedlings were especially high. Initially, these studies involved identifying fungal organisms most commonly associated with root diseased seedlings. By far the most common associated fungi were in the genus *Fusarium*. However, fungi of the closely related genus *Cylindrocarpon* were also frequently isolated from roots of diseased seedlings. Since isolations routinely involved surface sterilization to remove ectotrophic rhizosphere organisms, we assumed isolated organisms were actual colonizers of root cortical and stele tissues. Because of their relative abundance, work on pathogenicity and epidemiology was initially concentrated on *Fusarium* spp.

The object of this paper is to summarize findings regarding associations of *Cylindrocarpon* spp. with diseases of container-grown conifer seedlings at several nurseries in northern Idaho and western Montana. Descriptions will include types of diseases encountered, suspected inoculum sources, and attempts to ascertain pathogenicity of the associated organisms. Short descriptions of representative isolates are also included.

DISEASE CHARACTERISTICS

Root Decay of Western White Pine

A few years after initiating work on root diseases of container-grown seedlings, we encountered an important disease of western white pine *(Pinus monticola* Dougl.) seedlings. This disease was different from common root disease found in this and other conifer species because affected seedlings usually lacked above-ground symptoms such as chlorotic and necrotic foliage. Diseased seedlings were usually not discovered until they were extracted from containers at the end of the growth cycle prior to storage or outplanting. When extracted, affected seedlings displayed various levels of root decay, which was especially concentrated at the bottom of root plugs. Decayed roots lacked epidermal and cortical tissues. Although decayed roots were non-functional, seedlings with extensive decay still did not exhibit root disease symptoms. Apparently, while in the nursery, seedlings with root decay still had sufficient functioning roots required for absorption of necessary nutrients and moisture. This type of disease was restricted to white pine; other conifer species grown nearby were unaffected. Growers were especially concerned because remedial actions to reduce disease impact could not be taken because of the lack of symptoms. Isolations from decayed root systems consistently yielded *Cylindrocarpon* spp. For example, in one study (James and Gilligan, 1990), *Cylindrocarpon* spp. were isolated from all seedlings with severe or moderate root decay and from about 80% of all root pieces sampled. In comparison, *Fusarium* spp. were isolated from only about 5% of root pieces sampled. *Cylindrocarpon* spp. were also obtained from all sampled seedlings without root decay and from the interior surfaces of most cells from styroblock containers used to grow seedlings (James and Gilligan, 1988). Very small amounts of *Cylindrocarpon* were isolated from white pine seed of the same seedlots affected by root decay (James and Gilligan, 1990). Seedlings with severe root decay grew at the same rate and were the same size as those with moderate or slight root decay, making it extremely difficult for growers to locate affected seedlings prior to removal from containers.

This disease has occurred on white pine seedlings fairly consistently at several nurseries during the past few years. Attempts to reduce damage by drenching with fungicides have largely been unsuccessful (James *et al.*, 1988). Damage has been reduced after submerging containers in a hot water solution between crops (minimum temperature of 80^{o} C for 2-3 min.). This has greatly reduced levels of residual inoculum carried within both styroblock and plastic containers (James, 1989; James and Woollen, 1989). We have been unable to

consistently isolate *Cylindrocarpon* spp. from the soilless growing media most nurseries use (a mixture of sphagnum peat and vermiculite) (James and Gilligan, 1990). Therefore, we are unsure how the majority of inoculum is being introduced into affected container nurseries.

Currently, major recommendations for reducing damage from this disease involve hot water container treatments (James and Woollen, 1989), controlling irrigation to reduce overwatering which may increase host susceptibility to infection (Thornton, 1960), maintaining fairly acid conditions suppressive to *Cylindrocarpon* spp. (Holdenrieder and Sieber, 1992; Matturi and Stenton, 1964), and instituting sanitation procedures. Sanitation recommendations include removing diseased seedlings, surface sterilization of most surfaces (floors, benches, walls) within greenhouses between crops, and removal of organic debris within production spaces. Unfortunately, these efforts have not been completely successful at all nurseries; some nurseries still encounter high losses.

Because western white pine is in such high demand for reforestation throughout the northern Rocky Mountains, any level of reduced nursery production is important. Therefore, some seedlings with root decay are currently being accepted for outplanting because of high demand for stock. To determine effects of limited root decay on outplanting performance, studies were recently initiated to monitor stock at three different sites in northern Idaho. Survival, growth, and causes of mortality as well as persistence of *Cylindrocarpon* on root systems will be monitored on outplanted seedlings. This study should improve our ability to predict disease effects on outplanting performance and establish cull standards for seedlings that have poor survival potential.

Root disease of whitebark pine

Another interesting disease associated with *Cylindrocarpon* spp. was recently found on whitebark pine (*Pinus albicaulis Englem.*) being grown in containers (James, 1991). This conifer species is grown for grizzly bear habitats at high elevations in Montana. Although whitebark pine has not routinely been grown in nurseries, production recently increased because of greater need for stock to rehabilitate habitats. Seed germination was an initial problem, but was overcome by mechanically scarifying seedcoats to break dormancy. Seedlings produced in greenhouses grow slowly, requiring two year cycles to obtain seedlings of sufficient size for outplanting. Typical post-emergence damping-off associated with *Cylindrocarpon* spp. was sometimes found (James, 1991). However, once past the damping-off stage, survivors usually appeared disease free for the remainder of the first growing season. Root disease symptoms appeared during

the second growing season, often just before seedlings were due for shipment to the field. Typical root disease symptoms included tip dieback and needle chlorosis and necrosis (James, 1991). Root systems of affected seedlings were usually extensively decayed, similar to that found in white pine. Losses were often extensive, causing great concern.

Extensive sets of isolations were performed on crops grown during successive years. Results of these isolations consistently yielded high levels of *Cylindrocarpon* spp. In one study, *Cylindrocarpon* was isolated from 97% of all sampled seedlings with root disease symptoms as well as from 100% of the seedlings sampled that lacked disease symptoms (James, 1991). Most seedling root systems from both categories were colonized by these fungi. Other possible pathogens, such as *Fusarium and Pythium*, were found at very low levels. *Cylindrocarpon* spp. were common colonizers of plastic containers used to produce seedlings (James, 1989 and 1992); fairly high levels of seed infection was also found (James, 1991).

Losses from root disease of whitebark pine were reduced after hot water immersion treatments were instituted (James, 1991). However, some disease occurred periodically despite repeated application of fungicide drenches. Careful monitoring of moisture content and avoiding overwatering should help reduce disease severity (Dennis and Sutherland, 1989). Sanitation procedures, especially removal of diseased seedlings, should also help reduce losses. Since most seedlings are extensively infected even though they may not exhibit disease symptoms, improved control procedures should be geared toward reducing amounts of introduced inoculum and controlling seedling susceptibility to disease following root infection.

Miscellaneous damping-off and root diseases

Throughout work on root pathogens of container-grown conifer seedlings, we frequently isolated *Cylindrocarpon* spp., along with several other fungi, from damped-off and root diseased seedlings (James, 1988). In most cases, these fungi were less abundant than *Fusarium* spp. *Cylindrocarpon* spp. have also been routinely isolated from seedcoats of several conifer species and from styroblock and plastic containers (James, 1989; James and Gilligan, 1988). *Cylindrocarpon* spp. were usually isolated more frequently from the bottom of container cells where they persisted between seedling crops (Dennis and Sutherland, 1989; James, 1989).

In many cases, *Cylindrocarpon* spp. have been dismissed as probable secondary colonizers of roots or saprophytes that might colonize organic material in greenhouses (Booth, 1966; James, 1988). *Fusarium* spp. are often more

pathogenic, based on pathogenicity assays we have performed (James *et al.*, 1989). However, root decay of western white pine and whitebark pine root disease are two important diseases caused by *Cylindrocarpon* spp. in container nurseries in the inland Pacific Northwest. It is possible that *Cylindrocarpon* spp. act in conjunction with other root pathogens to cause major disease problems (Bloomberg and Sutherland, 1971; Duda and Sierota, 1987; Sluggett, 1972). Eliciting of disease by *Cylindrocarpon* spp. may be related to factors of host susceptibility affected by environmental conditions under which seedlings are grown (Beyer-Ericson *et al.*, 1991; Duda and Sierota, 1987; Rouatt *et al.*, 1963).

ASSOCIATED CYLINDROCARPON SPECIES

Most isolations from both diseased and non-diseased seedlings have yielded several species of *Cylindrocarpon.* We have used Komada's selective agar medium (Komada, 1975) for routine isolations. Occasionally we also make isolations onto V-8 juice agar amended with several antibiotics when water mold fungi *(Pythium* and *Phytophthora* spp.) are suspected. *Cylindrocarpon* isolates frequently grow well on Komada's medium, although not nearly as fast as some *Fusarium* spp., especially *F. oxysporum* Schlecht. Characteristic growth habit and pigmentation produced by common *Cylindrocarpon* spp. (see below) make rapid identification on Komada's medium possible. In most cases, single spore cultures are grown on potato dextrose agar (PDA) to confirm identification. Some cream-colored isolates of *Cylindrocarpon* may initially be confused with pionnotal forms of *Fusarium* on Komada's medium; however, single sporing onto PDA and carnation leaf agar (Fisher *et al.*, 1982) makes differentiation easier.

From root decayed white pine seedlings, most isolations yielded *C. destructans* (Zins.) Scholten, *C. didymum* (Hartig) Wollenw., and *C. tenue* Bugn. (James, 1987; James and Gilligan, 1990), based on species descriptions of Booth (1966). From root diseased whitebark pine seedlings, most isolates were classified as *C. destructans*, with a few identified as *C. tenue* (James, 1991). Most isolates from other conifer species examined and the majority from styroblock and plastic containers have been identified as *C. destructans* (James, 1989; James and Gilligan, 1988). One recent set of isolations from the roots of non-diseased white pine seedlings yielded several isolates identified as *C. cylindroides* Wollenw. (James, Dumroese and Wenny, unpublished).

The following are brief descriptions frequently used to separate the various isolated *Cylindrocarpon* species:

C. destructans:

Colony morphology: most isolates produce floccose aerial mycelium that becomes pale brown with time. The degree of brown to reddish-brown pigmentation produced on PDA varies (Samuels and Brayford, 1990). Some isolates produce deep brown pigmentation, whereas others may be more beige to light brown. Brown pigmentation is common on Komada's medium as well as on PDA.

Conidia: Both macroconidia and microconidia are produced by all isolates. The macroconidia vary in length and number of septations (Samuels and Brayford, 1990); they will also vary in level of curvature. Microconidia are usually unicellular and may or may not be abundant. All conidia are produced on terminal or lateral phialides that form on branched or unbranched conidiophores.

Chlamydospores: One major characteristic of this species is chlamydospore production, especially in cultures that are several weeks old. These chlamydospores may initially appear smooth and hyaline, but later they become characteristically brown and rough due to superficial deposits (Booth, 1966). Level of brown pigmentation increases in older cultures.

Teleomorph: The described teleomorph for this species is *Nectria radicicola* Gerlach & Nilsson (Booth, 1966; Samuels and Brayford, 1990). We have never found this perithecial state on any of our diseased seedlings or within greenhouses.

***C. didymum*:**

Colony morphology: Most isolates produce floccose, aerial mycelium that is beige to light brown (Booth, 1966). Older cultures may produce slightly darker pigmentation, especially with purple-grey tints. However, the colonies are never as dark brown as those of *C. destructans*.

Conidia: Both macroconidia and microconidia are produced. The macroconidia are fairly short with usually one or two septations. They are either straight or slightly curved. Microconidia are defined as single celled, although some may approximate the length of macroconidia. There is usually a gradation of

micro- into macroconida based on size (Booth, 1966). Conidia are produced in terminal or lateral phialides borne on branched or unbranched conidiophores.

Chlamydospores: Chlamydospores are commonly produced in chains or clumps, especially in cultures several weeks old. They are initially hyaline, but become light brown with age. They remain smooth and thick-walled.

Teleomorph: No known teleomorph has been described for this species.

C. tenue:

Colony morphology: Isolates are usually fairly uniform in morphology. They have been placed in this taxon primarily on the basis of conidial, condiophore and chlamydospore morphology. Our isolates are routinely cream colored on both PDA and Komada's medium, although they may become pale brown with age. Colonies may initially produce floccose to felted aerial mycelium. However, mycelium often becomes appressed as confluent sporodochia produce a pionnotal appearance.

Conidia: Only one type of conidium is produced. These are usually quite uniform in size and most have one septum dividing the conidium into two cells. They are uniformly straight and are produced mostly from simple lateral phialides borne alternately along condiophores.

Chlamydospores: Isolates will produce either solitary or chains of chlamydospores which are initially hyaline, but become slightly brown with age. They may have minute deposits on their surface, giving them a slightly roughened appearance.

Teleomorph: No known teleomorph stage has been described.

C. cylindroides:

Colony morphology: Isolates produce colonies with abundant white to slightly beige aerial mycelium. Older cultures may appear light brown in color. A

distinctive feature is the powdery appearance of colonies due to abundant production of microconidia.

Conidia: Both macroconidia and microconidia are produced. Macroconidia are usually one to several septate and straight or slightly curved. Microconidia are abundantly produced in most isolates and are borne at the end of phialides produced on penicillately-branched conidiophores.

Chlamydospores: No chlamydospores are formed in any isolates.

Teleomorph: The described teleomorph is *Nectria fuckeliana* Booth, but we have never found this perithecial stage on diseased seedlings or within greenhouses.

TESTING PATHOGENICITY

Extensive screening of a large number of *Cylindrocarpon* isolates for pathogenicity has not yet been done. Ideally, isolates obtained from root decayed seedlings should be screened for their virulence on that species. However, we have only screened 10 selected isolates for "general pathogenicity" in standard tests devised for testing virulence of *Fusarium* spp. The ten isolates tested included five identified as *C. destructans* and five as *C. tenue*. One test involved rapid evaluation of damping-off potential under typical greenhouse conditions and the other test was a rapid screening of damping-off in the laboratory. Each test evaluated effects of test isolates on Douglas-fir, the standard species used to screen pathogenicity of *Fusarium* isolates. We wanted to determine relative isolate aggressiveness using standard techniques to compare *Cylindrocarpon* isolates with those previously tested in the genus *Fusarium*.

Our greenhouse test involved incorporating inoculum consisting of cornmeal, PDA and perlite colonized by test fungi (Miles and Wilcoxon, 1984) into peat vermiculite growing media and sowing the media with Douglas-fir seed. We monitored seedling emergence and terminated the experiment when emergence was completed (based on germination in control, non-inoculated treatments). The test was completed in 21 days. Isolations were made from diseased seedlings with post-emergence damping-off, selected non-germinated seed, and a sample of non-diseased seedlings. We determined if the inoculated fungal isolates were responsible for disease, thus completing Koch's postulates.

Our laboratory test was similar, with individual young Douglas-fir germinants exposed to the same type of inoculum in glass vials. Young germinants were monitored daily for a maximum of 14 days to determine when (or if) disease occurred. Virulence of tested isolates was based on days of seeding survival, occurrence of disease, and recovery of the inoculated isolate from the germinant. A numerical rating system based on a maximum of 100 (all germinants killed in one day) was developed. In this way, we compared relative virulence of tested isolates.

Results from our greenhouse test indicated isolates of *C. destructans* reduced seedling emergence about 25%; those of *C. tenue* reduced emergence by 20%. Very low rates of post-emergence damping-off were noted, i.e., 4.0% and 3.0% for *C. destructans and C. tenue*, respectively.

Our laboratory test confirmed relatively low levels of virulence for the *Cylindrocarpon* isolates tested. The average virulence rating for *C. destructans* was 36.2, that of *C. tenue* was 30.9; overall rating for all *Cylindrocarpon* isolates tested was 33.6. This was low when compared with many *Fusarium* isolates similarly tested. For example, some isolates of *F. proliferatum* (Matsushima) Nirenberg have ratings in the mid-80s to 90s. Other *Fusarium* species are usually somewhat lower, but often in the 50s to 70s (James, unpublished).

Based on these limited tests, we concluded the *Cylindrocarpon* isolates tested were not very aggressive pathogens, at least causing damping-off to Douglas-fir germinants. We plan to expand pathogenicity testing of *Cylindrocarpon* spp. under conditions in which we see disease problems in container nurseries. There may be wide variability in the ability of selected isolates to induce disease (Dahm and Strzelczyk, 1987a). Some isolates may inherently be more pathogenic than others or may only be pathogenic under certain environmental conditions that affect host responses to infection (Dahm and Strzelczyk, 1987b; Rouatt *et al.*, 1963).

CONCLUSIONS

We have recently encountered *Cylindrocarpon* spp. frequently associated with root diseases of container-grown conifer seedlings at several nurseries in the inland Pacific Northwest. These organisms are particularly important in causing extensive root decay of western white pine and root disease of whitebark pine. They are also sometimes primarily responsible for damping-off and root disease of other conifer species. These observations confirm reports that *Cylindrocarpon* spp. can be important pathogens in forest tree nurseries (Chakravarty and

Unestam, 1987; Sluggett ,1972; Unestam and Beyer-Ericson, 1990; Vaartaja, 1967).

Although *C. destructans* has usually been implicated as the major pathogen of this genus associated with forest trees (Samuels and Brayford, 1990; Unestam and Beyer-Ericson, 1990; Vaartaja and Cram, 1956), some reports have implicated other species of *Cylindrocarpon* as pathogens. For example, *C. didymum* was recently associated with root lesions of declining cedar *(Chamaecyparis* sp.) (Hennon *et al.*, 1990), although this fungal species was unable to induce seedling mortality in inoculation tests. *Cylindrocarpon didymum* was also isolated from killed Scots pine seedlings along with *C. destructans* (Duda and Sierota, 1987). *Cylindrocarpon cylindroides* has been implicated as a root pathogen of *Abies* spp. (Lang, 1981) and frequently isolated from non-mycorrhizal roots of *Picea* (Holdenrieder and Sieber, 1992).

Control has proven difficult for diseases associated with *Cylindrocarpon* spp. Fungicide drenches applied when above-ground symptoms become noticeable are largely ineffective (James *et al.*, 1988). Some diseases, such as white pine root decay, do not routinely exhibit above-ground symptoms. Another important problem affecting control efforts is the high likelihood of root infection without either root decay or production of disease. *Cylindrocarpon* spp. may often be isolated at relatively high levels from surface-sterilized conifer roots (James, 1988). Factors affecting decay initiation, toxin production, or other pathologic behavior are unknown. Some *Cylindrocarpon* spp. or isolates of a particular species may be more pathogenic than others. We feel that there are certain conditions, most as yet undefined, during which these fungi become important nursery pathogens. They seem well adapted to greenhouse operations and are usually found in most conifer seedling crops.

Because of their continued importance as pathogens, increased research on epidemiology and factors affecting disease severity is needed. Such efforts should also include pathogenicity testing and work on population genetics of these fungi, especially as it relates to pathogenicity on conifer seedlings. When more is known about how these organisms function as pathogens, improved control recommendations can be developed.

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