ABSTRACT

Forest tree nursery seedling diseases in West Virginia and Pennsylvania, incited by *Cylindrocladium scoparium* have resulted in significant mortality of both hardwood and conifer species. This species of *Cylindrocladium* along with other plant pathogenic species of the genus are difficult, if not impossible, to control once they infest soil. Soil-borne propagules of *C. scoparium* can colonize leaves, pine needles, and woody materials present in the soil. These organic substrates provide food and sites for overwintering for these fungi. The cylindrocladia can move through the soil via water and by various cultural practices employed by nursery personnel. Soils within the nursery, not previously cultivated, usually do not possess *C. scoparium* unless they receive water from infested seedbed soils or soil containing the fungus. Low areas within a seedbed (infested with C. scoparium) usually have higher densities of the plant pathogen, thus seedlings in these sites are usually more symptomatic (diseased). Soil from infested nursery soil appears to be the primary way in which the fungus is spread throughout the nursery. Nursery personnel must take certain precautions within their nurseries once C. scoparium is detected in nursery soils.

CYLINDROCLADIUM: COLONIZATION, GROWTH, AND SURVIVAL IN NURSERY SOILS IN WEST VIRGINIA AND PENNSYLVANIA

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There are numerous species of *Cylindrocladium* which have been found to be pathogenic to a variety of economic crops in the United States and throughout the world (5, 12). One species which is extremely virulent and destructive to forest tree nursery seedlings is *C. scoparium* Morgan (1, 3, 4, 9). This fungus is often confused with *C. floridanum* Sobers and Seymour; however, it should not be since they are both the same species (5, 13). In this paper we will refer to all fungi of both species as *C. scoparium*.

For the past several years in state owned nurseries in West Virginia and Pennsylvania, *C. scoparium* has been responsible for nursery seedling diseases of numerous conifers and hardwood forest tree species. Two other species, *C. crotalariae* and *C. parvum* have also been recovered from soil and seedlings, but their numbers are few and they are not considered important in the disease complex at the nurseries. Species of *Fusarium* and *Cylindrocarpon* have likewise been associated with the nursery diseases at the nurseries (10, 13). Additional studies are ongoing to ascertain their role, if any, in nursery seedling diseases at the nurseries.

Control of *C. scoparium* in seedbed soils, once established, has been most difficult and usually not at all (2, 6, 10). Until control procedures have been definitively established, the only way to combat diseases incited by *C. scoparium* is to prevent the organism from becoming established in seedbed soils. This entails an understanding of many facets of this fungal plant pathogen's invasion, growth, and survival in nursery soils. To understand these factors, one must first be aware of the biology and physiology of *C. scoparium.* Hunter and Barnett (6, 7) established many of the cultural and nutritional factors through which *C. scoparium* and many other species of *Cylindrocladium* grow, sporulate, and produce microsclerotia.

Species of *Cylindrocladium* are much alike in their cultural characteristics with the most notable difference being the size of their conidia. C. scoparium was the first species of the genus to be named and it is the most important one in seedling diseases.

C. scoparium is a facultative parasite and is soil-borne. When conditions are favorable and a susceptible host is present, this fungus can become parasitic. The fungus overwinters as microsclerotia or mycelium on soil debris and in the spring new growth comes from the microsclerotia producing new hyphae.

This fungus has two distinct vegetative forms, a mycelial and a microsclerotial state. One to seven-day old cultures have white to tan, fluffy, aerial mycelium which often collapses after the seventh day of growth. Two-week old colonies of *C. scoparium* are often radically striate with concentric zonation, due to the formation of the microsclerotia. It is about the fifth day of growth that submerged hyphae begin to develop the distinct reddish-brown microsclerotia, starting at the center of the colony and progressing outward (5, 6). These

small, deeply colored, submerged bodies are formed through a process of bulbous enlarging and lateral budding of cells of a single hypha. The sclerotial precursors become massed into a spherical or oblong sclerotium or they may become chainlike. The microsclerotia often impart a ropy appearance to the thallus and a coarse texture usually results on the surface of the agar. The microsclerotia are the fungal propagules which are persistent in the soil, often for many years in a fallow field.

Under conditions of high humidity the fungus may produce a conidial stage. Hunter (5) has shown that cylindrical conidia are held together by a slime drop which gives the conidial heads a columnar appearance. Often the conidiophores possess a hypha with a swollen tip (vesicle) which extends above the conidia. Hunter (5,6) reported that several species of *Cylindrocladium* grew poorly at 12 and 32 C., but they all would grow well between 24 to 28 C. Even though species of *Cylindrocladium* do not grow well at 12 C., they can survive in culture and in soil at tempertures several degrees below 0 C. The cylindrocladia likewise survive pH's as low as 2.0 and as high as 11.0. Additionally these fungi can tolerate high osmotic pressures and high concentrations of some heavy metal compounds. Most soil-borne fungi are adversely affected or killed by the extreme of physical, chemical, and environmental factors which leave the cylindrocladia unaffected.

Knowing the biology of *C. scoparium* provides ways to develop techniques to recover the fungus from soil, seedlings, woody materials and other substrates. The inception of the geranium baiting procedure by Hunter et al. made it possible to qualitatively assay the soil for *C. scoparium* and other species of the genus (8). This procedure enables researchers to determine whether this fungus is in the soil even when the densities are extremely low. Nursery personnel and others will now be able to determine whether any propagules (most likely microsclerotia) of *Cylindrocladium* are present in the soil. When the fungus is discovered to be present, a quantitative assessment of the density can be made by a wet-sieving procedure used in conjunction with a selective agar medium (11). This procedure enables researchers to definitively establish the density of *C. scoparium* in soil and to make assessments of whether it is practical to grow forest tree nursery seedlings in the infested seedbeds.

The geranium baiting soil-bioassay for *C. scoparium* and other cylindrocladia has provided a very excellent way to assess seedbed soils for these nursery plant pathogens, even in the presence of the myriad of competing bacterial and fungal microorganisms. The major importance of this technique is in the visual identification of conidial fructifications on the leaf, thus isolation procedures need not be employed unless recovery is necessitated. Hunter et al. (8, 10) isolated numerous fungi from West Virginia and Pennsylvania nursery soils, many being plant pathogens; however, the presence of other fungi did not usually interfere with the colonization and microsclerotial/conidial production on geranium tissue in soil by *Cylindrocladium* spp. A few researchers have had success with alfalfa seedlings as baits for cylindrocladia in nursery soils in Minnesota, but Hunter for cylindrocladia in nursery soils in Minnesota, but Hunter (5) and others during the current research studies found alfalfa seedlings as poor baits for isolating *Cylindrocladium* spp. Other researchers have also used azalea leaves to bioassay for *Cylindrocladium* in soil in different nursery areas and reported successful results. Our studies with abscised azalea leaves and alfalfa tissue showed that these substrates were poor for conidial production while implanted in the soil, thus lessening their effectiveness as rapid identification techniques of *Cylindrocladium* spp. Our geranium baiting technique was utilized successfully at West Virginia and Pennsylvania nurseries to facilitate accurate *Cylindrocladium* soil surveys. *Cylindrocladium scoparium,* and for the first time, *C. parvum,* and *C. crotalariae* were recovered using geranium baiting and our selective medium. Our data are conclusive that *Cylindrocladium* is ubiquitous in and around the nurseries and regardless of whether the soil was fumigated. Repeated soil fumigations are common at the Parson's nursery, yet cylindrocladia populations seem unaffected. One reason could be predicated upon the cultural practices employed at the nursery. Straw and sand-sawdust mixtures are commonly applied and might alter the carbon/nitrogen ratio of the soil, thus favoring *Cylindrocladium* persistence and longevity through

increased microsclerotial production. However, *C. scoparium* was found in some samples tested at the perimeter of the West Virginia, but not in most areas of the Pennsylvania nurseries (non-fumigated and no added substrates).

When *C. scoparium* is in seedbed soils, it will ultimately invade and perhaps infect nursery seedlings. This fungus also enters many other soil substrates (leaves, pine needles, wood residues of seedlings, wood chips and sawdust) which can serve as food sources and as sites where the fungus can remain dormant for many years. Many of these soil materials are routinely added to seedbed soils as part of the cultural practices employed by nursery personnel. Thus, it is most important to ascertain whether *C. scoparium* is in the soil and to employ a selective medium to isolate it when it has invaded various wood substrates.

Glucose-lima bean rose bengal agar (GLRBA) proved to be an excellent medium for the isolation of *Cylindrocladium* species from diseased seedling tissue and other woody substrates and we believe it to be superior to other selective media previously used. Several workers used two percent malt extract agar as a selective medium for the isolation of *Cylindrocladium* species from diseased plant tissue, while others employed potato dextrose agar, acidified or not, for the same purpose. Bugbee and Anderson (1) plated surface-sterilized blue and black spruce sections on both two percent malt extract agar, with and without antibiotics, and potato dextrose agar. They reported that developing *C. scoparium* colonies were frequently overgrown by faster growing fungi. This problem was never encountered with GLRBA and we recommend, like Newhouse and Hunter (10), that it be adopted as the standard medium for assaying diseased seedling tissue for *Cylindrocladium* species.

Cylindrocladium scoparium was routinely isolated by Hunter et al. (8, 10) from diseased nursery seedlings with GLRBA. However, they found that the use of glucose-yeast extract agar containing rose bengal (GYRA), as the isolation medium, favored the recovery of *Fusarium* species from the same seedlings. We confirmed that yeast extract is a more favorable nutrient source than lima bean agar for the isolation of *Fusarium* species. Although *Cylindrocladium* recovery decreased on GYRA, many *Clindrocladium* species exhibit very good growth on glucose-yeast extract agar media. Since yeast extract is much richer in nitrogen and lima bean agar, the increase in available nitrogen, or the smaller carbon-nitrogen ration of GYRA as compared to GLRBA, may have allowed *Fusarium* species to colonize the medium faster than *Cylindrocladium* species. The *Fusarium* isolates produced dense aerial mycelia and sporulated abundantly on the GYRA medium, and all bacteria and contaminating fungi were effectively suppressed. For these reasons, we propose GYRA as an excellent selective medium for the isolation of *Fusarium* species from diseased plant tissue. Since certain species of *Fusarium* may be important in the disease complex it is vital to determine the presence of these fungi as well as cylindrocladia in nursery soils.

Cylindrocladium scoparium, *C. crotalariae*, and *C. parvum* are known to infest soils and infect seedlings growing in seedbed soils in West Virginia, Pennsylvania, and other areas of the United States. At this time, no economically effective control for *C. scoparium* has been established; therefore, it is essential to prevent this fungus from entering seedbed soils as well as preventing its spread in nursery soils once it has been found there.

Some suggested nursery practices are:

(1) Never use soils collected from seedlings which are being prepared for shipping, especially when *Cylindrocladium spp.* have been found in seedbed soils. These types of soils have been found to contain extremely high densities of *C. scoparium* at the Mont Alto nursery in Pennsylvania.

(2) When possible, limit the application of leaves and woody materials to seedbeds known to have pathogenic cylindrocladia. The inoculum potential of these fungi may increase fungi may increase and these substrates provide food sources and sites for overwintering and survival.

(3) Attempt to eliminate low areas within seedbeds. Recent studies of ours have shown that propagules of C. *scoparium* can be disseminated in seedbed soils via water and they will be deposited in higher numbers in lower areas of a seedbed than elsewhere.

(4) Always remove and burn all seedlings known to be severely infected (diseased) with any species of *Cylindrocladium.* Dead seedlings (under humid conditions) may become a substrate for the production of conidia by cylindrocladia. These conidia can be wind and water disseminated, thus the fungus can be spread widely throughout and become established in sections of a nursery not previously inhabitated by these fungi.

(5) Make certain that seedlings which are selected for shipping are disease-free. Check closely for lateral root infections (intermittent blackened areas) and for infections of the primary root at or near the soil-line. Diseased seedlings do not survive well when outplanted, especially on sites which are poorly prepared.

(6) It is essential that nurserymen know whether seedbed soils contain cylindrocladia. Nursery equipment (especially tractors) and personnel are probably the primary modes by which the cylindrocladia enter the soil and are subsequently disseminated throughout seedbed soils. Whenever a seedbed is found to contain high densities of C. *scoparium,* any equipment used there must be thoroughly cleaned to remove all soil. Failure to do so will probably result in the soil infestation of this fungal pathogen when the equipment is used in soil devoid of the fungus. Laboratory tests have proven this to be a likely way in which the fungus can be established in non-infected soils.

Seedling diseases incited by C. *scoparium* and closely related species are widely known in many areas of the world. Until accepted controls of these species are established, nursery personnel must take precautions to prevent soil infestation and dissemination. We believe that by following our suggestions, nurserymen will limit the invasion and spread of the cylindrocladia in nursery soils.

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