The Biology of Cylindrocladium scoparium in Wisconsin Forest Tree Nurseries

Walter G. Thies and Robert F. Patton

Former Research Assistant and Professor, respectively, Department of Plant Pathology, University of Wisconsin, Madison 53706.

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ABSTRACT

The primary propagule of *Cylindrocladium scoparium* in nursery soils was determined to be the microsclerotium. Microsclerotia apparently were incapable of repeated germination. Viability in soil samples was greatly reduced by drying. Viable microsclerotia were recovered in relatively high numbers from soil that had remained in clean fallow for 7 years. They were present in varying amounts in all blocks of a Wisconsin nursery, but were recovered only occasionally and in small numbers from soil within windbreaks in the nursery. The fungus was not isolated from soil of the forest adjacent to the nursery. A vertical gradient existed in nursery soil, the microsclerotial population increasing from the surface down to the plowline at about the 15-cm level. No microsclerotia were detected below the plowline.

The fungus did not grow through nonsterile soil, nor did it move from one seedling to another by root contacts.

The fungus was probably introduced on roots of infected seedlings in internursery stock transfers. Once introduced, it increased in infected roots and was distributed throughout the nursery by cultural operations. The microsclerotial population increased in soil when soybean was used as a cover crop, but was lowest in soil in which corn, grown as a cover crop, was incorporated. Phytopathology 60:1662-1668.

Cylindrocladium scoparium Morgan has been reported on plants in 66 genera of 31 families in many areas of the world, inciting such diseases as leaf spots, fruit rots, stem lesions, and root rots (12).

The fungus was responsible for serious losses in the late 1950's and early 1960's in Wisconsin State Forestry Nurseries. Mortality of 60-90% of the conifer transplants occurred in nurseries at Hayward, Rhinelander, and Wisconsin Rapids. Bugbee (3) isolated the fungus in 1961 from roots of black spruce (*Picea mariana*) and white spruce (*Picea glauca*) transplants from Griffith Nursery at Wisconsin Rapids. Major losses occurred with transplants of red pine (*Pinus resinosa*), the most commonly grown species.

Identification of *Cylindrocladium* species is based largely on the shape and size of the vesicle typically produced at the end of a sterile hypha that accompanies the conidiophore. Morrison (8) and Morrison & French (9) recently concluded that the fungus causing a serious root rot of conifer seedlings in nurseries in the Lake States, previously identified as *C. scoparium* Morgan, was morphologically identical with a new species described from peach in Florida, *C. floridanum* Sobers & Seymour (11).

The purpose of this research was to study the biology of the fungus causing severe transplant mortality in Wisconsin nurseries, including its distribution and survival in nursery soils. In our work, many thousands of propagules were isolated directly from the soil and germinated. The range in size and shape of vesicles in the resulting cultures was broad enough to include both *C. scoparium* and *C. floridanum*. We decided that until a more definitive study of the taxonomy of *Cylindrocladium* species was published, we would continue to use the name *C. scoparium* for the organisms we studied.

MATERIALS AND METHODS.—*Nursery site.*—The major portion of our studies was carried out at the Griffith State Forestry Nursery, Wisconsin Rapids, Wisc. The nursery, established on a Plainfield soil type, has been operated continuously since 1930. Cultural operations have resulted in a surface layer, approximately 18-25 cm in depth, which is high in organic matter. The interface, or plowline, between the dark upper layer and the light sand below is distinct. The nursery was partitioned by windbreaks into 11 blocks. Each block was further divided into sections by overhead watering pipes approximately 90-150 m long and spaced 18 m apart. Within each section, the trees were planted in 9 bedrows, each 1.2 m wide.

Detection of fungus in soil samples.—Spot-plate method.—Usual procedures for direct isolation of soil fungi from small samples proved unsatisfactory. Bugbee & Anderson (4) detected the fungus by planting alfalfa (*Medicago sativa* 'Vernal') in samples of field soil in the greenhouse. We modified the bioassay used by Bugbee & Anderson (4) to detect the presence of *C. scoparium* in small soil samples of about 0.5 cc or less (12, 13). Essentially the method or "spot plate" technique consisted of growing alfalfa seedlings in small soil samples as a bait for *C. scoparium* and then culturing the fungus from the seedlings on an agar medium.

Identification of *C. scoparium* was facilitated by the formation at room temp of distinctive and characteristic red-brown microsclerotia, often in concentric zones on common media such as potato-dextrose or malt extract agars. Conidia were produced sparsely, and thus could not be used consistently for positive identification. Instead, observations for the typical microsclerotia were made through the underside of the petri plate. Usually 10 subsamples were used to test a sample. Results were stated in terms of the number of subsamples from which the fungus was recovered.

Direct isolation process.—Quantitative estimates of

the relative number of propagules of *C. scoparium* in soil were made by a direct isolation process involving a refinement of washing and wet-sieving techniques (12, 14). The propagules of the fungus first were separated from most of the soil on the basis of size and density. This soil fraction was designated the inoculum conc. Then the propagules in the inoculum conc were allowed to germinate and grow on a selective medium. The details have been described previously (14). Results of the direct isolation process were stated in terms of p/g (propagules of *C. scoparium* per g oven-dry wt of the original sample).

Soil sampling techniques.—Our methods of sampling soil changed as our understanding of the ecology of the fungus grew. Three different types of samples were taken: mass samples, strip samples, and soil plugs.

A mass sample was a collection of soil from three locations in an area to be tested. Before collecting the sample, we removed and discarded the top 2.5 cm of soil. The sample was then collected with a trowel from the next 10 cm of soil.

Strip samples were collected at regular vertical intervals from the exposed face of a pit and were used in determinations of the number of propagules of *C. scoparium* at various depths in the soil. The soil collected was from strips approximately 0.6-1.3 cm thick, 10-13 cm wide, and 30-61 cm long at each of the various depths to be sampled. The first sample (S) was collected at the surface at one edge of the pit. Then soil was removed and discarded from beneath the S sample down to a depth of 5 cm. At the 5-cm level, a second strip sample was collected. This was repeated at each 5-cm interval down to a depth of 35 cm. In a variation of this technique, samples were collected from only the S, 7.5-cm, and the 15-cm levels.

Soil plugs were collected with a 2.5-cm diam tube-type soil sampler pushed vertically into the soil. Each sample consisted of a collection of 10 cores of soil. That portion of the soil from below the plowline was discarded. Three types of samples were collected: spot, line, and transect samples. A spot sample consisted of 10 plugs collected randomly within an area no more than 0.5 m sq. A line sample consisted of 10 plugs taken at 20-cm intervals along a straight line. Transect samples were comprised of a series of line samples collected at 6-m intervals along a diagonal across a nursery section. Each of the 10-19 line samples in a transect was kept separate and tested individually.

Soil samples were stored at 4 C in tightly closed plastic bags. Sampling times and assay trials were coordinated so that samples were not stored for more than 30 days.

RESULTS.—*Identification of the soil-borne propagule.*—Identification of the primary propagule of *C. scoparium* in the soil was a logical consequence of the direct isolation process. While developing this process, we determined which of several soil fractions contained the most propagules. Unblended soil was washed successively through 100-, 200-, and 325-mesh nematode screens. Materials that collected on each screen, and those that passed through the 325-mesh screen, were collected as separate fractions. Each fraction was tested for propagule content as in the direct isolation process: the fraction was dispersed in dilute water agar, mixed into melted and cooled (46 C) NPX medium (14), poured into petri dishes, and incubated at room temp.

Colonies of *C. scoparium* developed from materials that collected on the 100-, 200-, and 325-mesh screens, but not from materials that passed through the 325-mesh screen. The propagules were neither conidia nor small mycelial fragments, since these could have passed through the 325-mesh screen. Ninety-five per cent of the colonies of *C. scoparium* developed from materials that passed through the 100-mesh screen but were retained on the 200-mesh screen.

Propagules of *C. scoparium* were located by examining the inoculated plates of NPX medium every 6 hr under oblique illumination with a dissecting microscope. Every developing colony was removed to another plate for further growth, and its initiating particle was mounted on a slide for identification by microscopic examination. Finally, the initiating particles of colonies that were eventually identified as *C. scoparium* were compared with microsclerotia from a 12-day-old culture of *C. scoparium*. Of over 400 propagules that yielded *C. scoparium*, approx 100 were examined microscopically, and all were determined to be essentially the same as the microsclerotia from the reference culture. About 10% of the *C. scoparium* cultures were associated with pieces of organic matter, and these also emerged from microsclerotia in or on the organic matter particles.

Repetition of spot-plate tests.—Presence of roots of susceptible alfalfa seedlings in the soil in the depressions in spot plates apparently induced microsclerotia of *C. scoparium* to germinate. Hyphae from the germinated microsclerotia then infected the roots. Whether such germination and subsequent infection could occur more than once by propagules in a given soil sample was examined by testing twice the subsamples with the standard spot-plate technique. After the alfalfa seedlings from the first test were removed and before the soil could dry, each subsample was immediately replanted with three alfalfa seeds and treated as in a standard spot-plate test.

Of 720 subsamples, 193 yielded *C. scoparium* in the original test, while only 9 yielded the fungus in the retest.

Effect of drying on microsclerotia.—The effect of drying of the original soil sample was examined by the spot-plate technique. One group of subsamples from a soil sample was allowed to dry in the spot plates for 2 hr before they were watered and another group was kept moist. As time permitted, this experiment was performed with samples involved in various tests in the nursery. In every trial, the number of previously dried subsamples from which the fungus was recovered was not more than one-third the number of constantly moist subsamples that yielded *Cylindrocladium*.

The effect of air drying of soil samples was also tested by the direct isolation process. A mass sample from the field was thoroughly mixed and divided into

two approximately equal portions. One was tested immediately. The other portion was left in an open bag in the laboratory for 2 or 3 days before being tested. Since the purpose of the test was to determine whether or not precautions should be taken against drying of samples exposed to the air, no attempt was made to insure that water was lost evenly from the whole sample. The experiment was repeated several times with different field samples. In every trial, soil samples that were allowed to dry yielded only 20-30% of the number of viable propagules obtained from samples not allowed to dry.

The effect of prolonged air drying of field soil on viability of propagules was studied. A mass sample of field soil known to contain a high concn of the fungus was divided into two samples. Each was spread out in a pan to air dry for 5 days, then placed in a plastic bag that was left open in the laboratory for 3 months, after which the samples were tested with the direct isolation process. After drying, the samples had a moisture content of 1% and assayed only 1 p/g. The original inoculum level was not calculated in terms of p/g, but samples collected later from the same area yielded a propagule concn of 15-20 p/g.

Effect of time on viability of microsclerotia in soil.—The number of viable microsclerotia in naturally infested soil that had lain in clean fallow for 7 years was determined. A small plot of ground in Griffith Nursery had the following history: (i) used for nursery production from fall 1955 to spring 1961: (ii) high mortality of red pine in this area; (iii) no crops on the area (including cover crops) since spring 1961; (iv) biweekly cultivation had kept the land weed-free; (v) no biocides were used on the plot. During the summer of 1968, strip samples were collected from the S, 7.5-cm, and 15-cm levels at four sites in the plot and tested by the direct isolation process. At all four sites, the number of microsclerotia increased from the S to the 15-cm level, and the average concn detected were S — 11 p/g; 7.5 cm — 16 p/g; and 15 cm — 34 p/g.

Location of microsclerotia in soil.—Vertical distribution was determined with tests made both by the spot-plate technique and the direct isolation process. For the spot-plate tests, strip samples were collected from approximately 90 sites in the nursery at 5-cm intervals from the surface down to 60 cm below the surface. From the last 40 sites, samples were collected through only the 35-cm level. The fungus was recovered from fewer subsamples from samples collected at the surface than from samples collected below the surface. The fungus was recovered regularly from samples at the 20-cm level, occasionally from samples at the 25-cm level, but never from samples collected below 25 cm.

For assay by the direct isolation process, strip samples were collected from approx 25 sites in the production areas of the nursery. Samples were collected from the S, 7.5-cm, and 15-cm level and from 2.5 cm below the plowline. At every site tested, the number of microsclerotia increased from the surface down to the 15-cm level. Populations as high as 59 p/g were detected at the 15-cm level. No viable microsclerotia were detected from samples collected 2.5 cm below the plowline.

Horizontal distribution was determined in the nursery as well as in soil of the forested area immediately adjacent to it. Tests with the spot-plate technique on three mass samples collected in each block of the nursery proved the presence of *Cylindrocladium* in every block. Quantitative differences in microsclerotial populations along a section transect were determined by the direct isolation process with samples collected from five sections. The concn of microsclerotia of *C. scoparium* was not uniform within a section, as indicated by the range of the number of microsclerotia in the individual line samples making up the transect of the section. Thus, the ranges in the five sections examined were 7 to 16 p/g, 17 to 49 p/g, 18 to 59 p/g, 0.06 to 30.00 p/g, and 1 to 35 p/g.

The population of microsclerotia beneath the overhead watering pipes, an area not used for production, was compared with that in adjacent production areas. Pairs of mass samples were collected along several watering pipes, one sample of the pair under a pipe and the other 1.2 m away in the adjacent production area. Although the fungus was recovered by the spot-plate technique from every sample taken beneath a watering pipe, in every case the number of recoveries was less than in the corresponding sample from the production area.

Soil under windbreaks was also assayed. Within several windbreaks adjacent to production areas known to have high populations of microsclerotia, 24 mass samples were collected. The fungus was not detected by the spot-plate technique in any of these samples. Later six of the areas were resampled, and the 18 samples were tested by the direct isolation process. *Cylindrocladium scoparium* was detected in only 2 of the 18, and the populations in these (0.6 p/g and 0.03 p/g) were not more than 2% of those in the adjacent production areas.

Finally, mass samples were collected from the forested area west of the nursery, at points about 45 m from nursery production areas that contained high populations of *C. scoparium*. The fungus was not detected in 20 samples tested by the spot-plate technique or in 6 samples tested by the direct isolation process.

Movement of the fungus through the soil.—Growth of hyphae through soil was tested in petri dishes. Soil was sterilized in petri dishes by exposure to propylene oxide in a closed container for 2 weeks, then aerated for 3 weeks. Six dishes each of nonsterile and of sterilized soil were inoculated in the center with approx 200 microsclerotia, and another group of six each were inoculated with three actively growing colonies. Microsclerotia were screened from 14-day-old agar cultures, and colonies used for inoculum were from shake cultures grown for 5 days from spores in Czapek's medium. Noninoculated dishes served as checks. After incubation at room temp for 10 and 20 days, sample plugs of soil were removed with sterile plastic straws and tested on spot plates. Two samples were collected

overlapping the inoculated spot, and four samples 1 cm from the center along four equally spaced radii.

After the 10-day incubation, *C. scoparium* was recovered from the inoculated points of all dishes, confirming that both mycelial and microsclerotial inocula were viable. On the surface of sterile soil inoculated with mycelial colonies, white mycelium was observed, and *C. scoparium* was recovered from the soil plugs collected 1 cm from the center of four of the six dishes. The fungus was not recovered 1 cm from the center in dishes containing nonsterile soil, however, or in dishes containing either sterilized or nonsterile soil inoculated with microsclerotia. Similar results were obtained with dishes incubated 20 days and also when the experiment was repeated.

Growth along pine roots.—The ability of *C. scoparium* to move from one plant to another along their interlocking root systems was determined by inoculating soil at one end of short rows of transplants. Twelve red pine seedlings several months old grown from seed in flats of sterilized soil were transplanted in rows with roots of adjacent plants overlapping and touching into sterilized soil in cardboard containers. In mid-July, 2 weeks after transplanting, inoculum of *C. scoparium* obtained by washing and chopping shake-grown colonies in a blender was poured into a hole in the soil at one end of the line of transplants in four of the cartons. Two cartons were left noninoculated as checks. After incubation outdoors for 5 weeks, soil samples were removed with sterile plastic straws and tested with the spot-plate technique. Root systems of the transplants were examined and selected root pieces were cultured.

The inoculum was viable when used, but the fungus could not be recovered from soil around the inoculated spot. Some roots of each transplant adjacent to the inoculum were rotted and *C. scoparium* was isolated from these, but not from any of the other transplants.

Possible sources of introduction of the fungus.—Two possible sources of introduction of the fungus were investigated: the peat amendment periodically added to the nursery beds; and infected seedlings. Samples of peat collected from the peat storage piles were tested by either the spot plate technique or the direct isolation process, but the fungus was not detected in any of the samples.

Since shipments of seedlings or transplants between nurseries are commonly made, infected seedlings are a possible source of new infections. Red pine seedlings of various ages from 2 weeks to 3 years were excavated, the roots were washed and examined, and selected root segments were cultured on acidified Czapek's agar. Approximately 4,000 trees were examined, although records were not kept on every individual tree. Of 100 2-week-old seedlings from which isolations were attempted, 76 yielded *C. scoparium*. In the seedbeds, many seedlings at the end of their first and second growing seasons had root rot but showed no top symptoms. Approximately 60% of the 2-year-old seedlings were infected. After this stock was transplanted in the spring, many of the transplants died from root rot later in the season, and an estimated 25% of the remaining healthy-appearing transplants at this same time had *Cylindrocladium* root rot.

Effect of excised pine roots on amount of inoculum.—A considerable amt of root material is left in the soil during the lifting of stock. The effect of such material on the population of microsclerotia was studied. A 10-g portion of roots from healthy red pine transplants was added to each of five pots containing 450 g of infested soil. Another five had no roots added. All 10 pots were incubated from 5 to 6 months from spring to fall in a nursery shed and watered as necessary to keep them moist. At intervals during the last month of incubation, paired samples including one pot with added roots and one check pot were evaluated for the microsclerotial populations by the direct isolation process. The pots to which roots were added had fewer microsclerotia, averaging 10.5 p/g, than the check pots, which averaged 15.6 p/g. The difference was significant at the 1% level according to "Student" t-test.

Effect of cover crops on amount of inoculum.—Since cover crops were a possible substrate for the buildup of *C. scoparium* in the soil, the effect of plowing-under a cover crop on the population of microsclerotia was determined in a pot experiment. Infested soil from the nursery was collected, screened, and thoroughly mixed, and 450 g (396 g dry wt) were put into each of 35 pots. Cover crops were grown for 5 weeks in infested nursery soil in flats; then the roots were washed free of soil and blotted free of excess moisture before being added to the pots of soil. Five lots of each of four cover crops were so prepared for each of the following treatments: 10 g flax/pot; 20 g soybeans/pot; 20 g oats/pot; 10 g corn/pot; 20 g corn/pot; and 40 g corn/pot. A check pot with no amendment was also prepared. The pots were then arranged into seven replicates, each containing one pot of each cover crop addition plus a check pot with no amendment. The crop plants were not broken up as they were added by folding or mixing into the pots of soil. The pots were stored, watered, and processed in the same way and at the same time as those in the pine root experiment described previously.

Populations of microsclerotia in the soil after the cover crops had decomposed during the 5- to 6-month storage period are shown in Table 1. Populations in the soil amended with soybean were extremely variable, ranging from none to 251. Those in the pots in which corn was incorporated, however, were all consistently low. All plates made from soil in which corn or flax had decomposed contained 5 to 10 times as many total fungal colonies as plates made from soil in which oats or soybeans had decomposed, and there were more total colonies on the plates from the 40 g/pot corn treatment than from the 10 g/pot corn treatment. *Trichoderma* spp. and *Fusarium*-like colonies were predominant. Similar results were observed in isolations when excised pine roots were added to the soil. The fact that the treatment means were not statistically different in an analysis of variance is probably explained by the great heterogeneity shown by the soybean data. When Dun-

TABLE 1. Populations of *Cylindrocladium scoparium* microsclerotia after various cover crops had decomposed in the soil over a 5- to 6-month period

		Cover crop incorporated into each pot[a]					
Replicates	Check	Flax (10 g)	Oats (20 g)	Soybeans (20 g)	Corn (10 g)	Corn (20 g)	Corn (40 g)
1	8	5	9	251	1	1	0
2	4	4	5	1	0[b]	0[b]	0
3	11	5	2	0	0	0	0
4	8	5	11	0	2	0	0
5	15	11	18	88	0	3	0

[a] Data as propagules/10 g.
[b] *Cylindrocladium scoparium* isolated but population averaged less than 1 p/10 g.

can's multiple range test was applied, the mean of the soybean treatment (68 p/10 g) was significantly different from the mean of each of the other six treatments at the 5% level. In preliminary trials, use of cover crops had not increased propagule populations significantly. There were indications, however, that corn and possibly also flax reduced the population markedly.

Ability of microsclerotia to infect red pine roots.—The ability of microsclerotia to germinate and infect at various locations on the roots of red pine was investigated by inoculating roots with inoculum conc. Six 2-year-old red pine seedlings raised in sterilized soil in the greenhouse were removed, washed, and replanted in sterile sand in root observation boxes with part of the root system placed against the removable glass wall of the box. The seedlings were transplanted 2 months before inoculation and were watered regularly with Hoagland's solution. Inoculum conc was prepared the day it was used from soil containing a high population of *C. scoparium*. Four seedlings were inoculated at five sites, one seedling at four sites, and one seedling was left noninoculated as a check. Roots were inoculated by removing the glass side and placing a small quantity of inoculum conc on a 2- to 5-mm segment of the root at each site. Five days after inoculation, a 5- to 7-mm segment of root was removed at each inoculation site as well as 3- to 5-mm pieces adjacent to the inoculation sites. Root pieces were washed, surface-sterilized in 1% sodium hypochlorite for 1 min, washed in sterile water, plated on NPX medium, and incubated for 10 days at room temp. The fungus was recovered from 21 of the 24 inoculated sites, from all sizes of inoculated roots including root tips to segments of 3-mm diam. Also, four of the pieces proximal to the inoculation sites and one distal to the inoculation site yielded the fungus. It was not isolated from roots of the check tree.

DISCUSSION.—The horizontal distribution of the fungus in the nursery, and our investigations of possible sources of its introduction, indicated that it was introduced on plant stock and spread by cultural operations. Although Barron (2) isolated *C. scoparium* from forest soils in Ontario, he noted that it is recorded infrequently from soil, and M. P. Backus (*personal communication*), in 20 years of studying soil microflora in Wisconsin, has never recovered *C. scoparium*. Freter & Wilcoxson (6) isolated the fungus from cultivated fields in Minnesota, and Scholten (10) obtained it from red pine in the Knife River Nursery near Two Harbors, Minn., but seedlings in adjacent forest soils were not infected. We were unable to isolate the fungus from forest soil adjacent to Griffith Nursery. Recovery of only a few propagules from soil under windbreaks in the nursery suggested that propagules might have been blown there from adjacent highly infested production areas.

Colonies of *C. scoparium* developed from microsclerotia which were screened directly from soil and cultured on a selective medium. No conidia or mycelial fragments were observed as colony sources. In experiments on vegetative growth in soil, no evidence was obtained that the fungus has the ability to permeate the soil as viable mycelium. Although conidia produced on aerial portions of plants are viable for a few weeks in a moist atmosphere, they become nonviable quickly in a dry atmosphere (1, 4) and are killed after 36 hr at 0 C. We concluded that if conidia or mycelium are present in the soil at all, only microsclerotia are important in overwintering the fungus or sustaining it from one crop to the next.

Germination of microsclerotia apparently is induced by contact with roots or root exudates of a susceptible host. Spot-plate tests indicated that such germination occurred only once. Viability of microsclerotia and hyphae that grew from them was lost between the time of removal of seedlings from the first test and development of new roots from the second sowing of alfalfa. Other explanations are possible, as the microsclerotia were not examined visually, but it seems likely that germination as a result of an initial stimulus exhausted the microsclerotia. In the absence of a suitable substrate for colonization, the developing hyphae then died.

Increase in microsclerotial populations in nursery soils apparently occurs on two main substrates: (i) infected roots left in the soil; and (ii) susceptible cover crops plowed under as green manure. Microsclerotia were observed in the cortex of red pine roots during this work, and also in diseased spruce roots by Bugbee & Anderson (4). Failure of the fungus to colonize soil or healthy excised pine roots added to soil indicated that it has low competitive saprophytic ability, and that uninfected plant parts such as roots removed in the lifting operation do not constitute a source of increase. In preliminary cover crop experiments, populations of microsclerotia apparently did not increase. In the main experiment, however, exceptionally high popu-

lations were detected in two of the replicates of soybean amendment. Soybean is an extremely susceptible host, and since the plants used as the amendment were grown in naturally infested soil, it is possible that rapid invasion of roots of some of the plants by *C. scoparium* resulted in the production of large numbers of microsclerotia in advance of colonization by saprophytes. Wisconsin nurserymen recognized the possible danger of using soybeans and stopped using them as a cover crop in the early 1960's. In earlier work in Wisconsin (J. Berbee & R. Johnson, *unpublished data*), the fungus was isolated consistently from soybean residue in infested soil. Conidia were regularly produced on infected soybean stem and leaf pieces incubated in a moist chamber. The results of our trials are conflicting, and more definite experimental evidence on the effect of soybeans on increase of inoculum must be obtained. Large-scale plot trials in the nursery to obtain such evidence are now in progress.

Suppression or reduction of the population of microsclerotia to very low levels in soil amended with corn plants suggests the possibility of biological control. Our results agree with those of Scholten (14) whereby the addition of corn as organic matter reduced the number of plants infected in infested soil. Similarly, Green (8) found that the incidence of *Verticillium* wilt of peppermint was markedly reduced following continuous cropping sequences of corn. He suggested that germination of microsclerotia of *Verticillium albo-atrum* apparently occurred in the rhizosphere of corn, but little or no subsequent colonization of the root system took place. Although the mechanism of the reduction of *C. scoparium* propagules is unknown, the high colony counts on assay plates suggested that the decrease might be due to competition or antagonism by associated microorganisms favored by certain amendments.

The vertical distribution of microsclerotia appeared as a gradient of increasing concn from the surface down to the plowline. No microsclerotia were recovered 2.5 cm or more below the plowline, whereas up to 59 p/g were recovered 2.5 cm above the plowline, suggesting that the max depth from which the fungus could be recovered depended on the depth to which soil was mixed by plowing. Since drying reduced the germinative ability of microsclerotia, many of those brought to or near the surface by plowing, disking, and other cultural operations would lose viability, thus resulting in a gradient of increasing concn with depth down to the plowline.

Where moisture is available, microsclerotia can remain viable in host-free soil for many years. The population remained relatively high in the area in Griffith Nursery kept in clean fallow for 7 years, and Morrison (8) recovered a few viable microsclerotia from a nursery that had been out of production for 8 years. Anderson (1) noted that the fungus remained viable for a year or more in soil that had been inoculated. Thus, clean fallow cannot be recommended as a control measure. The fungus evidently remains in a viable but inactive state in the soil until stimulated to germinate by some agent. The requirements for germination of the microsclerotia, however, have not been precisely defined.

Although seedlings are infected by *C. scoparium*, under our conditions they suffer less mortality than transplants. The reason lies in the response of seedlings to attack. Growth of seedling root tips apparently results in encounters with microsclerotia with resulting infection and death of the root tip. The fungus moves slowly in a root, and the response of the seedling is a proliferation of roots above the diseased area, as noted by Cox (5) and confirmed by our observations. The infected root dies back from the tip to older tissues where the fungus appears to be stopped in some manner (5). In a heavily infested soil, all of the roots may be infected eventually and seedlings die, but in a soil with a lighter infestation, the seedlings may strike a balance, developing a top commensurate with the functional portion of the root system.

With transplants, however, more drastic reduction of the root system is favored by two additional factors: (i) Roots of transplants are severely pruned to make transplanting easier; and (ii) transplanting may result in placement of large roots in contact with microsclerotia. If infection of large roots occurs, as our data from the inoculation experiment indicated, the large roots may die, resulting in consequent death of many smaller subsidiary roots. In soils with a high population of microsclerotia, much or all of the root system may be so killed, making death of the plant extremely likely from inability of the reduced root system to supply water to the top. Indeed, during periods of moisture stress, the damage from *Cylindrocladium* root rot is greater than in seasons with abundant moisture. On infected transplants that are not killed, sudden browning and loss of older needles and a stunting of the current season's shoots and needles occurs, symptoms that could be produced by a period of rapid transpiration in plants with a reduced root system.

Although we have little knowledge of the population of microsclerotia necessary to cause mortality, it is conceivable that a lower population could cause mortality of transplants than would be required for death of seedlings. A single infection on a seedling might kill only one root tip, whereas a single infection on a large root of a transplant could cause death of many root tips.

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