Effect of Plant Residue Amendments and Chemical Treatments Upon the Inoculum Potential of Cylindrocladium floridanum in Soil

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ABSTRACT

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Microsclerotia of *Cylindrocladium floridanum* germinated more frequently in the vicinity of all plant amendments tested than in nonamended soil. The fungus exhibited characteristics of a competitive soil saprophyte and was capable of colonizing all buried crop amendments tested in four soils. Residues of plants susceptible to root infection by *C. floridanum* and propylene oxide-sterilized plant residues were colonized most frequently. Buried grass significantly increased the inoculum potentials of *C. floridanum* in field plots located on two different soil types. Ground corncobs did not affect the inoculum potential of *C. floridanum* in plots on one soil type. Trichlorodinitrobenzene and methyl bromide treatments prior to infestation of soil with *C. floridanum* had little effect upon *C. floridanum* inoculum potentials in amended plots.

Additional key words: microsclerotia, fungistasis, trichlorodinitrobenzene, methyl bromide.

Cylindrocladium floridanum Sob. and Seymour (formerly *C. scoparium* Morgan) causes an important root rot of conifers in nurseries of Minnesota, Wisconsin, and Michigan (1, 8, 9, 15). In most forest nurseries, cover crops are rotated with conifer stock, and these cover crops could affect the severity of Cylindrocladium root rot. A previous paper (8) indicated that actively growing cover crop grasses such as oats, rye, and wheat could significantly increase the inoculum potentials of *C. floridanum* in nursery soil. When cover crops are plowed under, they represent organic amendments which are known to alter populations of some soil-borne pathogens (13, 16, 17, 18).

Several workers have attempted to measure the effects of amendments upon the soil populations of *Cylindrocladium* spp. Thies and Patton (15) added crop residues to pots containing soil infested with *Cylindrocladium* spp. Their data, although inconclusive, indicated that soybeans could increase the *Cylindrocladium* population in the soil whereas corn and possibly flax might decrease it. Prey (10) reported that *Cylindrocladium* grew rapidly through beds with plowed-under soybeans. Sholten (11) found that green manure and peat seemed to enhance movement of *C. scoparium* through the soil, but ground corncobs and corn roots and shoots all reduced *C. floridanum* root rot of black spruce in the greenhouse. Thus, the possibility exists that cover crop amendments could result in either biological control or increased disease.

Our study was designed to examine the saprophytic colonization of cover crop residues by *C. floridanum* and to elucidate the effects of grass and corncob amendments on inoculum potentials of *C. floridanum* in nurseries.

MATERIALS AND METHODS

Crop materials were collected on 17 October 1968 from standing cover crops in the field which were representative of natural plant materials which would be plowed under and become available as substrates for soil microorganisms. Eight cover crops were used in this experiment (Table 1). Plant material was stored in plastic bags and frozen until used.

Germination of microsclerotia near cover crop amendments.—The technique developed by Morrison (9) for measuring the susceptibility of *C. floridanum* to fungistasis was utilized to estimate the effect of cover crop amendments upon germination of *C. floridanum* microsclerotia. A nursery soil (Omega sand) collected in June from the Cloquet Nursery, Forest Research Center, Cloquet, Minnesota, was stored at 4 C until needed. The soil was adjusted to 30-40% maximum water-holding capacity (5) which was equivalent to approximately 14% moisture content on a dry weight basis. Green leaves and stems of each cover crop, an equivalent of 0.5 g of dry material, were cut into pieces, and mixed with 20 g of soil. Soil plus amendments then were placed in petri dishes and compacted with a spatula. Washed microsclerotia (9) were placed upon the surface and incubated at 28 C. Microsclerotia also were added to soil without crop amendments. Samples of all microsclerotia used were 100% viable on water agar. Three replicates were prepared for each crop. A similar technique was employed with a second set of cover crops, except that these crop amendments were allowed to decompose in the nursery soil for 24 days at 22-25 C prior to the application of microsclerotia. Germination of the microsclerotia was determined by examination after 48 and 96 hours. Each microsclerotium that produced one or more hyphae was considered germinated.

Saprophytic colonization of cover crop amendments.—Knife River sand from the Knife River Nursery, Two Harbors, Minnesota, and a sandy loam mixture [silt loam plus sand (3:1, v/v)] were used in this experiment. The Knife River soil was naturally infested with *C. floridanum*, and the sandy loam mixture was infested with *C. floridanum* by mixing it with Knife River soil (5:1, v/v). A portion of each of these soils was treated with 0.91 kg of methyl bromide in a 13.6 m^3 fumigation chamber for 8 hours. The two methyl bromide-treated soils were then mixed with artificial *C. floridanum* inoculum (Table 2) at a ratio of 20:1. The Knife River sand and the sandy loam mixture were adjusted to 40-50% maximum water-holding capacity (approximately 18 and 30% moisture content, respectively, on a dry weight basis) (5). *C. floridanum* inoculum potentials were determined for each soil by using the quantitative alfalfa assay technique (8). With this technique the percentage of vials with *C. floridanum*-infected alfalfa seedlings from a total of 100 1-2 g vials of soil represent inoculum potentials.

To determine the ability of *C. floridanum* to colonize crop residues, green leaves and stems of each cover crop, equivalent to 2.5 g of dry material were cut into pieces, approximately 1-cm square, subjected to propylene oxide fumes in an airtight container for 48 hours, and mixed with 180 g of each of the four soils. Similar amounts of natural amendments that had not been treated with propylene oxide were added to the soils in an identical manner. The soils plus amendments were placed in 15 cm diameter glass petri dishes, incubated at 28 C, and maintained at a stable moisture content. Two replications were prepared for each treatment and cover crop combination for each soil.

Twenty-one days later, 25 samples of each crop per replication were removed. These samples were washed in distilled water, treated with a 1% sodium hypochlorite solution for 30 seconds, rinsed twice in sterile distilled water, and placed on 2% malt agar containing 30 μg/ml aureomycin. The plated samples were incubated for 21 days at 28 C before being examined for *C. floridanum*.

Field plots.—In the spring of 1967, field plots 2-m square were established on a Waukegan silt loam in St. Paul, Minnesota. The following season, 1968, a similar experiment was established on Omega sand at the Cloquet Nursery. Plots were arranged in rows separated by 1-m divider strips. Plots at each site either received no treatment or were treated with a water spray containing a fungicide, trichlorodinitrobenzene (Chemagro 2635, 7% wettable powder) equivalent to 22.5 kg/ha, or methyl bromide (Dowfume MC-2, 98% methyl bromide and 2% chloropicrin), at the rate of 0.91 kg/4 m^2. Trichlorodinitrobenzene is active against some root-rotting organisms, but in initial tests it did not prevent root rot caused by *C. floridanum* in black spruce [*Picea mariana* (Mill.) B.S.P.] seedlings. Each plot treated with methyl bromide was covered with a 0.102-mm (4-mil) plastic tarp until 5 days following fumigation.

To establish *C. floridanum* in the plots following the chemical treatments, the soil in each plot was mixed with soil from the Knife River Nursery, which was naturally infested with *C. floridanum*. One group of three plots, each plot with a separate soil treatment (trichlorodinitrobenzene, methyl bromide, or nontreated) received plant residue amendments and one group of plots at each location served as a nonamended control. All plant residues were buried to a depth of 10 cm and thoroughly mixed with soil by hand. Plant residues were added to the soil at St. Paul on 27 June 1967 and the soil at Cloquet on 29 May 1968. Plots at St. Paul received approximately 550 g (wet wt) of fresh grass manure from the edge of the field. An effort was made to restrict this green manure to a single *Setaria* sp. A similar amount of an *Agropyron* sp. green manure was added to the plots at Cloquet. Only leaves and stems were used; root matter was excluded. A second amendment of 1,800 g of ground corncobs was added to a separate set of three plots in the nursery soil at Cloquet.

To eliminate the effect of root exudates from rooting grass residue, the plots with buried grass were periodically weeded and the soil was turned over again on 12 August at

TABLE 1. Germination of *Cylindrocladium floridanum* microsclerotia after 4 days near fresh and partially decomposed cover crop amendments in a natural sandy nursery soil

Cover crops	Microsclerotia germination per residue (%)[a]	
	Fresh amendments[b]	Partially decomposed amendments[c]
Soybeans	85 a	56 a
Oats	78 ab	51 ab
Setaria sp.	72 bc	54 ab
Clover	74 abc	19 cd
Corn	71 bc	39 bc
Buckwheat	66 bc	51 ab
Wheat	65 bc	39 bc
Rye	63 c	28 bcd
No crop	45 d	39 bc

[a]Values were derived from three replicates; 100 microsclerotia were counted per replication. Values in each column followed by the same letter are not significantly different $P = 0.05$.

[b]Amendments were cut from fresh standing crop material on 17 October and frozen until used.

[c]Crop material was treated in the same manner as fresh amendments except it was buried in nursery soil for 24 days before use.

TABLE 2. Percentage colonization by *Cylindrocladium floridanum* of natural and propylene oxide-treated cover crop residues buried in naturally infested Knife River soil[a]

Cover crop	Plant samples colonized[a] (%)	
	Propylene oxide[b]	Natural
Soybeans	76	52
Clover	58	46
Buckwheat	16	38
Oats	52	6
Corn	26	0
Setaria sp.	12	0
Rye	10	0
Wheat	0	2

[a]Values were determined as the percentage of 25-1 cm^2 pieces of leaf tissue from which *C. floridanum* could be isolated. Crop residues were cut from fresh standing crop material on 17 October and frozen until used. Each treatment was replicated twice.

[b]Plant samples were exposed to propylene oxide fumes in an airtight container for 48 hours.

St. Paul and on 14 July and 22 August at Cloquet. All plots were sampled periodically from June to October for *C. floridanum* inoculum potentials by using the quantitative alfalfa assay technique (8).

Determination of fungistasis, and the populations of fungi, bacteria, and actinomycetes.—The method used for determining fungistasis was that of Morrison (9) described above.

Populations of fungi, bacteria, and actinomycetes were estimated at intervals in plot soils. Standard serial dilution plate techniques were employed for these tests. Isolation media (4) were Allen's soil extract agar for bacteria and actinomycetes and peptone dextrose agar plus rose bengal and aureomycin for fungi.

RESULTS

Germination of microsclerotia.—After 4 days, more microsclerotia of *C. floridanum* had germinated in soil that had received fresh amendments of all eight cover crops than in nonamended soil (Table 1). Greater germination of microsclerotia occurred near fresh amendments than near partially decomposed amendments. Of crops that had been allowed to decompose for 24 days, only soybeans significantly increased germination of microsclerotia above that in nonamended soil. Germination near soybean residues that had been allowed to decompose for 24 days was significantly greater than germination which occurred near similarly treated corn, clover, wheat, and rye residues (Table 1).

Saprophytic colonization.—*Cylindrocladium floridanum* was capable of colonizing all of the buried cover crop residues tested in this experiment in all four soils. Colonization in all four soils was similar to that shown for the natural Knife River soil (Table 2). Soybeans, clover, buckwheat, and oats , in general, were colonized more often than plant residues of *Setaria,* corn, rye, and wheat. Propylene oxide-treated amendments were colonized to a greater degree than nontreated residues. The amounts of tissue colonization in a particular soil was correlated ($P = 0.05$) to the *C. floridanum* inoculum potential in the soil. Conidia and microsclerotia of *C. floridanum* commonly were observed on cover crop amendments (Fig. 1).

Seasonal variation of Cylindrocladium floridanum inoculum potentials in field plots.—The ability of *C. floridanum* to infect host plants fluctuated during the growing season in agricultural and nursery soils irrespective of the soil treatments, cover crops, or amendments involved (Fig. 2 and 3). At two locations in Minnesota, the highest inoculum potentials occurred in June or July and again in late September or October, and the lowest occurred during August or early September.

Effect of amendments and chemical treatments on Cylindrocladium floridanum inoculum potentials in field plots.—The effect of buried grass amendments upon inoculum potentials of *C. floridanum* in the Waukegan silt loam is shown in Fig. 2. The inoculum potential in plots containing buried grass was significantly higher ($P = 0.05$) than those in plots without amendments. Similar results were obtained for the Omega sand forest nursery

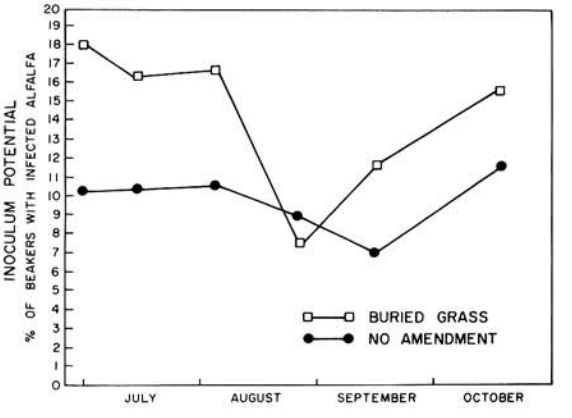

Fig. 2. The effects of buried grass amendments upon seasonal inoculum potentials of *Cylindrocladium floridanum* in a Waukegan silt loam from St. Paul. Numbers shown are averages from methyl bromide, fungicide, and nontreated plots. Plots were infested with *C. floridanum* by the addition of naturally infested soil on 27 June.

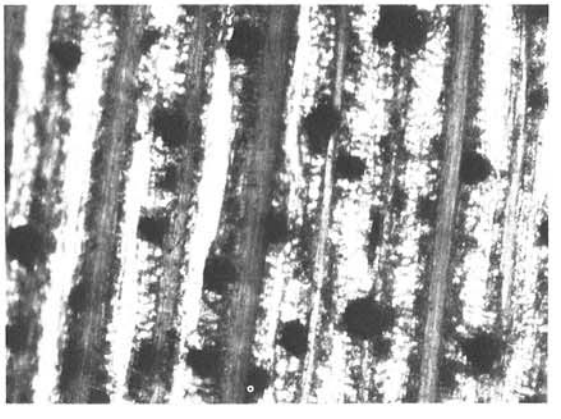

Fig. 1. Microsclerotia of *Cylindrocladium floridanum* embedded in decomposing leaf of *Setaria* spp.

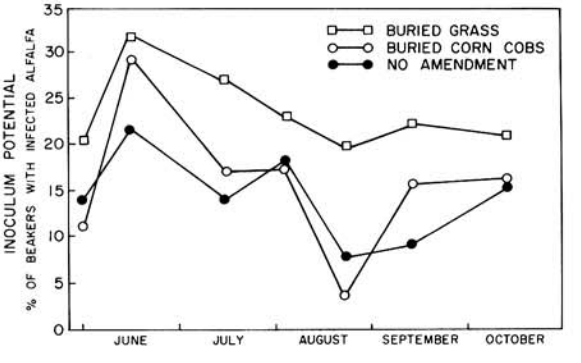

Fig. 3. The effect of buried grass and corncobs upon seasonal inoculum potentials of *Cylindrocladium floridanum* in Omega sand nursery soil from Cloquet. Numbers shown are averages from methyl bromide, fungicide, and nontreated plots. Plots were infested with *C. floridanum* by addition of naturally infested soil on 29 May.

soil (Fig. 3). Inoculum potentials in plots containing ground corncobs were not significantly different ($P = 0.05$) from those in unamended soil (Fig. 3). Neither methyl bromide nor trichlorodinitrobenzene treatments, prior to infestation with *C. floridanum* and incorporation of plant residues, significantly affected ($P = 0.05$) inoculum potentials of *C. floridanum* in field plots in either soil.

Effect of amendments and chemical treatments on germination of Cylindrocladium floridanum microsclerotia in field plots.—Germination of microsclerotia of *C. floridanum* did not appear to be affected by buried plant residues in the field plots. High germination of microsclerotia occurred in July, when inoculum potentials of *C. floridanum* were at their peak. The percentage of germinating microsclerotia decreased considerably throughout the remainder of the summer and autumn. In comparison, inoculum potentials of *C. floridanum* did decrease in August, but they increased again in September or October.

The only chemical which affected germination of *C. floridanum* microsclerotia was trichlorodinitrobenzene. It significantly ($P = 0.05$) reduced the average seasonal germination by 44%. Greatest inhibition of germination occurred early in the season, when the inoculum potentials of *C. floridanum* were low in fungicide-treated plots. Despite the low incidence of germinating microsclerotia, *C. floridanum* inoculum potentials eventually rose to levels higher than those in nontreated plots.

Effect of amendments and soil treatments upon populations of soil fungi and bacteria in field plots.—Grass amendments significantly increased fungus populations in the Omega sand (Table 3). Fungus populations increased in amended plots in June and remained higher than populations in nonamended plots throughout the summer.

Chemical soil treatments did exert an effect upon fungus populations in amended nursery soil (Table 3). Treatment of the Omega sand nursery soil with methyl bromide and subsequent addition of natural soil and amendments stimulated greater numbers of fungi than could be found in similarly treated plots that were not fumigated. Fungi in amended methyl bromide-treated plots increased abruptly, reached a peak in June or July, and then declined. Soil fungi in nonamended, methyl bromide-treated soil had the highest populations in August. In all methyl bromide-treated plots, the inoculum potentials of *C. floridanum* decreased suddenly, following the rise in population of soil fungi. Fungicide-treated soils in both amended and nonamended plots possessed populations of fungi that were not significantly different from populations in nontreated soil. Fungus populations in nontreated soil fluctuated randomly during the growing season, indicating a more or less stable population.

Bacteria and actinomycete populations were not significantly different in amended, chemically treated, or control plots and they could not be correlated with the inoculum potentials of *C. floridanum* in those plots.

Effect of soil types on C. floridanum inoculum potentials.—Inoculum potentials of *C. floridanum* generally were lower in the Waukegan silt loam than in Omega sand nursery soil (Fig. 2 and 3). Environmental conditions did not appear to be responsible. Similar results and further explanations were presented in an earlier publication (8).

TABLE 3. The effects of chemical treatments and organic amendments upon the fungal numbers in field plots in the Omega sand forest nursery soil from Cloquet throughout one growing season

Soil treatment	Fungal colonies, $\times 10^3$/g oven dry soil[a]			
	Buried grass	Buried corncobs	Nonamended	Mean[c]
Fungicide	81.0	157.6	50.0	96.2
Methyl bromide	701.3	160.3	81.0	314.2
No treatment	112.0	100.0	57.0	89.7
Mean[b]	298.1	139.3	62.7	...

[a]Values are derived from three sampling periods from June to October, each with two replications per sampling period.
[b]LSD ($P = 0.01$) = 131.4.
[c]LSD ($P = 0.01$) = 131.4.

DISCUSSION

Schroth et al. (12) stated that "germination and growth of *Fusarium solani* f. *phaseoli* in soil are primarily contingent upon the balance between fungistatic factors and the supply of organic nutrients." Results presented here indicate that these two factors are responsible for the behavior of *C. floridanum* in soil as well. The fact that the microsclerotia of *C. floridanum* germinated in the vicinity of several different crop residue amendments is not unusual. Lockwood (6) pointed out that fungistasis can be annulled by many amendments, and that germination is dependent not upon the presence of host tissue but upon the availability of suitable nutrients.

A competitive soil saprophyte is a fungus "with a summation of physiological characteristics that make for success in competitive colonization of dead organic substrates", whereas a soil-inhabiting fungus is capable of surviving indefinitely as a soil saprophyte (3). Garrett (3) stated that a fungus must invade dead organic material buried in nonsterilized soil before it can be called a soil inhabitant. Since *C. floridanum* does successfully colonize plant tissue under these conditions it would appear that *C. floridanum* may be called a soil-inhabitant and a good competitive saprophyte on two natural soils under conditions of this experiment.

The fact that *C. floridanum* is a good competitive saprophyte explains why addition of farm manure can increase Cylindrocladium root rot in nursery soil (D. O. Prielipp, 1969. *Unpublished* interim report on *Cylindrocladium* research. Kimberly-Clark Corporation, Norway, Michigan). These results also verify Prey's contention (10) that *C. floridanum* grew rapidly through nursery beds containing plowed-under soybeans and substantiate the data of Thies and Patton (15) which indicate that soybean plant residues can increase populations of *Cylindrocladium*.

Cylindrocladium floridanum was capable of colonizing all the cover crop amendments tested. However, only soybeans, clover, buckwheat, and oats are susceptible to root infection by *C. floridanum* (2, 7). Plant residues from these crops appeared to be colonized to a greater degree

than were other crops which were not susceptible to root infection by *C. floridanum* (Table 2). *Cylindrocladium floridanum* is pathogenic to leaves of wheat and rye (2, 14), and these crops were also colonized in our experiment (Table 2), but to a lesser degree. Corn, which is not thought to be a host for *C. floridanum* (12, 14), was also colonized. Even ground corncobs initially appeared to stimulate inoculum potentials of *C. floridanum* (Fig. 3) so available nutrients appeared to be adequate in all plant residues tested.

The germination of *C. floridanum* in the vicinity of plant residue (Table 1), the colonization of plant residues in nonsterile soil (Table 2), and the increased inoculum potentials of *C. floridanum* in soil amended with plant residues (Fig. 2 and 3) indicates that *C. floridanum* is able to multiply in soil in the absence of living host tissue. If *C. floridanum* is a soil inhabitant and can increase in soil without host plants, this could explain the difficulties involved with eradicating the fungus from infested soil. Not all of the survival capabilities of this fungus may be due to the resistant microsclerotia as often has been implied (7, 9, 10, 11, 15). Because *C. floridanum* can be a good competitive saprophyte, caution should be exercised when adding organic matter to soil known to contain *C. floridanum*. Plowing under cover crops or adding green manure to nursery soil can result in increased inoculum potentials of *C. floridanum*.

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