

## Survival of *Colletotrichum graminicola* in Soil

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### ABSTRACT

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Mycelia and conidia of *Colletotrichum graminicola* lysed when buried between nylon-mesh disks in field soil in petri dishes. At 16, 24, and 30 C, nearly all conidia were destroyed when incubated in soil for 14 days. At 8 C, 3% of the conidia were viable after 100 days of incubation in soil. Destruction of mycelium was complete after 16 and 11 days when soil was placed on mycelial mats or on cultures growing on peptone agar,

respectively. Cultures kept in darkness for 15 days did not lyse. Conidia were most abundant 8 days after addition of soil. However, sporulation of lysing mycelium did not appreciably enhance survival of the pathogen. There was no lysis of conidia or mycelium in sterilized soil. Sporulation of *C. graminicola* occurred concurrently with lysis of the mycelium.

*Additional key words:* anthracnose, soil invader.

Little is known about the survival of *Colletotrichum graminicola* (Ces.) G. W. Wils. in soil. The organism was not considered an important pathogen of corn in the United States before 1972 (12). In Indiana, *C. graminicola* is most important as the cause of anthracnose leaf blight and stalk rot of maize (*Zea mays* L.). The disease may develop at any time during the growing season; the pathogen may attack any part of the plant and can cause seedling blight, crown rot, root rot, stalk rot, leaf blight, top dieback, or kernel infection (11,12). Anthracnose is more severe when cloudy, warm, and humid weather occurs with abundant rainfall in July and August.

Survival of *C. graminicola* in maize residue was studied on naturally infected (6,8) and artificially inoculated residue (9). The fungus survived as stromatal tissue on maize stalks on the soil surface (9) without producing sclerotia or other resting structures. Root infection was obtained in the greenhouse and field by planting maize kernels in soil containing naturally infested stalk fragments, or with *C. graminicola* conidia (9). Crown and root rot occurred on seedlings derived from anthracnose-diseased kernels in the greenhouse and field (11).

Root and crown infection of maize originated from residue colonized by the pathogen (7). Thus, infested plant residue in the soil is probably the primary source of inoculum of *C. graminicola* attacking maize. Conditions that enhance microbial activity and hasten decomposition of infested maize residue, such as moisture, aeration, and warm temperature, were detrimental to survival of *C. graminicola* (9) because propagules were exposed directly to the soil environment.

This paper reports the saprophytic activity and lysis of *C. graminicola* propagules in soil in the absence of residue. A preliminary report was published (10).

### MATERIALS AND METHODS

**Survival of conidia in soil.** Unsterile Fox silt loam (pH 6.3; organic matter 2.5%; P<sub>2</sub>O<sub>5</sub>, 8 µg/g; K<sub>2</sub>O, 94 µg/g; NO<sub>3</sub>-N, 69 µg/g; and Mg 86 µg/g) was used. No anthracnose lesions developed on susceptible seedlings planted in the test soil. Prior to use, the soil was air-dried, passed through a 5-mm sieve, mixed by hand and divided into portions for treatments. The soil mixture was maintained at or near the moisture holding capacity (-1/3 bar [oven-dry weight basis]) by periodic weighing and watering. The drying boundary of the soil was determined by using a Haines

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apparatus (2) and in a pressure membrane apparatus with nitrogen as the compressing gas (7). The drying boundaries of the soil at or near the moisture-holding capacity and permanent wilting point were  $-1/3$  bar and  $-15$  bars, respectively.

Experimental procedures involving isolates of the pathogen, preparation of inoculum and soil plates, soil assay methods, and experimental conditions were previously described by Jooste (3). To simulate burial of conidia in soil, both halves of a petri plate were filled with soil and a suspension of *C. graminicola* spores was atomized onto a nylon disk over the soil surface. To simulate spores on the soil surface, only the lid was filled with soil and a spore suspension was atomized onto a disk. The bottom half of the petri plate was kept empty. The two parts of the petri plate were reassembled and placed in incubators at various temperatures. After incubation, the two parts of the petri plate were separated, a spore print was made by pressing strips of sterile acidified potato-dextrose agar (APDA) pH 5.0 (8) against the nylon disk and infested soil surface. The strips were mounted on glass slides, stained with lacto-fuchsin, and examined microscopically to determine the number of conidia per  $\times 100$  field. The final reading was an average of 10 fields per agar strip, two prints per plate and three replications per treatment. Agar strips were also incubated in a moist chamber for 12 hr before staining with lacto-fuchsin to determine the viability of conidia. Spores were considered germinated when their germ tubes were one-half the length of the conidium.

To determine the effect of soil matrix water potential on survival of conidia, Fox silt loam ( $-1/3$  bar) in petri plates was infested, incubated at 22 C, and assayed as described above after 2, 5, 8, 11, 14, and 18 days. Five plates were enclosed in boxes to prevent evaporation. The lid was removed from 10 plates to allow the soil to dry ( $-15$  bars). Sterile distilled water was added to five of these plates at 2-day intervals for alternate wet and dry cycles ( $-1/3$  bar to  $-15$  bars) and the remaining five plates were allowed to dry continuously.

Growth and development of conidia were determined with conidia obtained from plant residue and from lesions on green plants naturally infected under field conditions. Infested stalks with typical black stromata were surface disinfected and placed in a moist chamber for 48 hr. Conidia produced on the surface of disinfected corn stalks were washed from the surface. Conidia also were washed from sporulating lesions on green plants. The spore suspension was pipetted onto the surface of unsterile soil plates and assayed as described earlier. The petri plates with soil were incubated in plastic boxes to prevent evaporation and stored at 22 C with the soil matrix water potential at  $-1/3$  bar.

**Survival of mycelia in soil.** The lytic effect of soil on mycelia of *C. graminicola* was determined with a modification of the soil-plate techniques of Lockwood (4) and Jooste (3) by placing mycelial mats instead of spores between soil surfaces in petri plates. Petri plates containing 20 ml of 1% peptone agar (Difco, Detroit, MI 48232) were inoculated with *C. graminicola* and incubated at 30 C for 3 days; the cultures were covered with either moistened ( $-1/3$  bar) sterile or unsterile soil. The soil-covered petri plates then were incubated in moist chambers at 22 C. After 12 days, soil was washed from the surface of the agar and three 5-mm-diameter plugs of the colony were stained with lacto-fuchsin and flattened between two microscope slides for microscopic examination. The percentage of lysed mycelia was determined by counting the number of hyphal strands crossing the periphery of the field in treated and control plates. Controls were isolates of *C. graminicola* grown on peptone agar, without soil added. Fragmented or lysed hyphae did not absorb stain and were not counted. Three fields per agar plug, and three plugs per plate with three replicates were made for each treatment.

The effect of soil on lysis of *C. graminicola* was determined by placing 12-, 22-, and 30-mm-diameter mycelial mats between soil layers in petri plates. Flasks containing 250 ml of Fries' medium (8) were inoculated with *C. graminicola* and incubated in darkness for 6 days to produce mycelial mats with few or no conidia. Mycelial mats were removed and washed six times with sterile distilled water. Both halves of the petri plates were filled with soil and one of

each mat size was placed between the soil surfaces. The petri plates were reassembled, incubated in darkness at 23 C, and maintained at field moisture capacity ( $-1/3$  bar). In addition, five mats of each size were placed on oatmeal agar containing streptomycin sulfate and tetracycline-HCl (100 ppm each) (OMAA) to obtain initial viability of the mycelia. Also, five mats of each size were oven-dried for 24 hr at 70 C to determine their initial weight. Viability of mycelium and weight loss due to lysis were determined at intervals up to 25 days after incubation.

## RESULTS

**Survival of conidia in soil.** The effect of soil on viability of *C. graminicola* was studied by placing conidia on soil interfaces. Conidia lysed rapidly in soil at 16, 22, and 30 C, and slowly at 8 C. Fourteen days after incubation at 16, 22, and 30 C, nearly all of the conidia had lysed. However, after 100 days at 8 C, 3% were still viable (Table 1). Disrupted or lysed conidia occasionally were observed on agar strips before 14 days.

The agar strip method was used to determine the sensitivity of *C. graminicola* conidia to soil fungistasis. No germination of conidia occurred on natural soil, but nearly 90% germinated after 36 hr on sterilized soil.

Survival of conidia was affected adversely by alternate drying and remoistening of soil. Conidia were not recovered after three drying and remoistening cycles (Fig. 1), but conidia survived in

TABLE 1. Survival of conidia of *Colletotrichum graminicola* in petri-plates of unsterile soil during incubation at different temperatures

Time (days)	Number of viable conidia <sup>a</sup>			
	8 C	16 C	22 C	30 C
0	38	38	38	38
2	33	27	26	23
5	26	19	17	12
8	19	12	9	6
12	18	8	4	1
14	18	2	0	0
20	19	0	0	0
65	12	0	0	0
80	8	0	0	0
100	3	0	0	0

<sup>a</sup>Average of 10 microscope fields ( $\times 100$ ) per spore print, two prints per plate, three replicates per treatment. (The experiment was repeated three times.) Conidia were placed on a nylon mesh disk between the two halves of petri plates filled with unsterile soil kept at soil matrix water potential of  $-1/3$  bar.

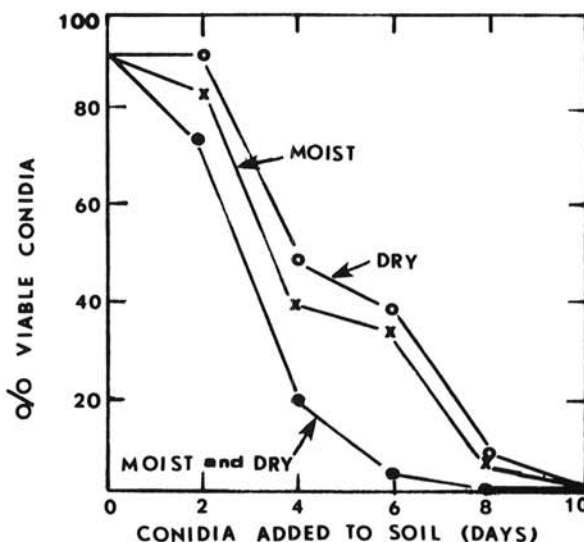


Fig. 1. Survival of *Colletotrichum graminicola* conidia in soil at 22 C and at matrix water potentials of  $-1/3$  bar (moist) to  $-15$  bars (air dry).

continuously dry or moist soils for 10 days.

**Survival of conidia from natural and artificial origins.** The percentage of germination of conidia from lesions on green plants was slightly higher than those obtained from dead residue. Conidia from dead residue survived 19 days and conidia from green plants survived 24 days at 22 C (Fig. 2). The number of conidia from both sources declined rapidly after 6 days. There appeared to be no selective advantage for survival of conidia produced from either the parasitic (green plant) or persisting phase (nonliving host residue).

**Survival of mycelia in soil.** Lysis of *C. graminicola* mycelia covered with sterile and unsterile soil were compared. Lysis of mycelium in unsterile soil was evident after 1 day, and the mycelia were completely lysed after 11 days (Table 2). All the mycelia covered with unsterile soil had disappeared after 16 days. However, mycelia covered with sterile soil were not affected. Numbers of hyphal strands were about equal to those observed in the agar control. The fungus did not produce chlamydo spores or sclerotia in the soil plate, although bulbous cells within hyphal strands were common.

Sporulation in unsterile soil occurred before lysis of mycelia. Conidia were most abundant 8 days after soil treatment (approximately 500 spores per square millimeter of agar surface). Sporulation did not occur on the peptone agar control grown in darkness, nor in peptone agar cultures covered with sterile soil. Results were similar when the test was repeated.

**Survival of mycelia mats in soil.** Dry weight of mycelial mats grown in Fries' medium decreased when placed in petri plates

containing unsterile soil. The loss of weight was due primarily to lysis of mycelia, but some loss may have been caused by leaching of nutrients from the mats. The growth or viability of mycelia in mats incubated on soil and then placed on OMAA decreased with time of incubation in unsterile soil. At 7 days, the percentage of mats with viable mycelium decreased for all sizes and at 15 days *C. graminicola* could not be isolated (Table 3). The 12-mm-diameter mat lost viability first, followed by the 22- and 30-mm mats. No lysis of hyphae or weight loss occurred up to 25 days when mats were placed in sterile soil.

*Trichoderma* spp., *Fusarium* spp., and actinomycetes were isolated from the mycelial mats with increasing frequency as the incubation time increased. Direct parasitism of *C. graminicola* by these fungi was not observed.

## DISCUSSION

Pathogenic soil fungi vary in their competitive saprophytic ability, growth through soil in competition with other soil microflora, and persistence in soil in the absence of a host. Lockwood (5) reported that mycelium from many different pathogenic fungi was destroyed when living or killed 4-day-old cultures were covered with field soil for 1-2 wk. Our data on the lysis of mycelia and conidia of *C. graminicola* agree with those of Lockwood (5). However, low temperatures simulating winter conditions tended to enhance survival of free conidia or mycelia in soil as well as mycelia in plant residue (Table 1). Conditions that reduce microbial activity or slow decomposition of plant residue favor survival of the pathogen. Information regarding this phase of the ecology of *C. graminicola* is of importance in developing effective control of the root-, crown-, and stalk-rot phase of the disease caused by this pathogen. Tillage practices that incorporate infested residue in the soil aid in hastening decomposition of residue and decrease the survival potential of *C. graminicola* (9).

Some *Colletotrichum* spp. produce sclerotia and survive for long periods in soil (1). Because *C. graminicola* does not produce sclerotia or other resistant structures and because conidia or mycelia in soil are lysed in a short period, this lysis may account for the effectiveness of deep or fall plowing and crop rotation in controlling the pathogen.

Sporulation occurred on agar plates covered with unsterile soil and was most abundant after 8 days of incubation. The conidia produced in soil plates were not lysed immediately, nor did they germinate in situ. After 11 days at temperatures of 16 C or above, the mycelium was totally lysed when covered with unsterile soil (Table 2). Conidia produced under these conditions extended viability of the pathogen to about 60 days. Soil-induced sporulation is not uncommon among soil fungi. The critical point is whether the mycelia lyse before they form conidia or resting structures. In our studies, conidia of *C. graminicola* were produced before lysis. Conidia derived from mycelia in decomposing residue may be a source of inoculum for root and stalk infection (9), but

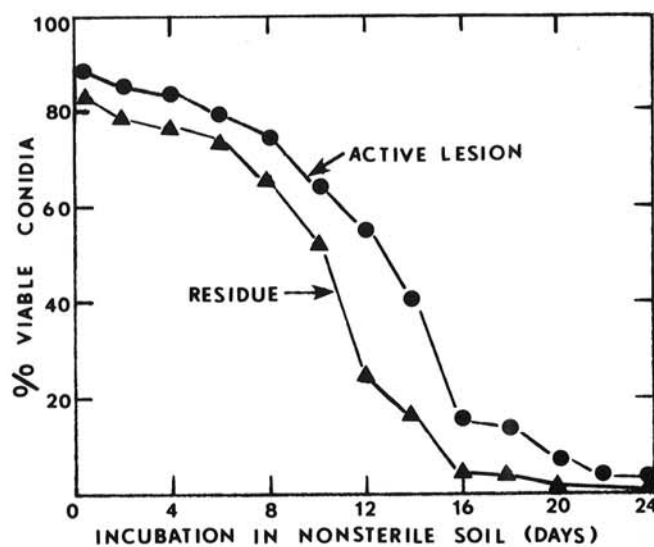


Fig. 2. Survival of conidia of *Colletotrichum graminicola* derived from plant residue and active lesions. Conidia were washed from plant residue and sporulating lesions and pipetted on the surface of unsterile soil at 22 C.

TABLE 2. Lysis of living mycelium of *Colletotrichum graminicola* on peptone agar cultures covered with unsterile or sterile soil

Time (days)	Number of viable hyphae <sup>a</sup>			% Lysis <sup>c</sup>
	Control <sup>b</sup>	Sterile soil	Unsterile soil	
1	121	134	110	9
3	122	139	85	31
5	120	141	53	56
8	114	136	16	86
11	117	137	0	100

<sup>a</sup> Average of six microscope fields (×100) per plate, three replications per treatment, the test was repeated three times.

<sup>b</sup> Petri plates containing 20 ml of 1% peptone agar inoculated with *Colletotrichum graminicola* and incubated under similar conditions without soil added.

<sup>c</sup> Percentage of lysed hyphae in unsterile soil based on number of viable hyphae in control.

TABLE 3. Dry weight and viability of mycelial mats of *Colletotrichum graminicola* covered with unsterile soil

Time (days)	Dry wt (mg) <sup>a</sup> of mycelial mats with diameters: <sup>a</sup>			Viability (%) of mycelial mats <sup>b</sup> with diameters:		
	12 mm	22 mm	30 mm	12 mm	22 mm	30 mm
0	18	55	90	100	100	100
2	18	51	86	100	100	100
4	14	45	76	100	100	100
7	9	39	68	66	83	83
10	8	34	67	33	66	50
12	8	35	64	0	33	17
15	6	33	57	0	0	0
20	5	27	50	0	0	0
25	4	25	45	0	0	0

<sup>a</sup> Average of replicated mats, the test was repeated two times. Mycelial mats were obtained by growing the fungus on Fries' medium in the dark at 23 C.

<sup>b</sup> Mycelial mats were washed free of soil and plated on acid oatmeal agar plus antibiotics.

conidia or mycelia free in soil are not considered effective primary sources of inoculum. Frequent sporulation on overwintering residue could account for increased inoculum density available for infecting the new corn crop.

Our results provide evidence that *C. graminicola* conidia or mycelia survived less than 20 days in soil at temperatures at 16 C or above. This suggests that the fungus is a soil invader and its survival is dependent on colonization of plant residue before its incorporation in soil (9,10). *C. graminicola* probably does not have a true saprophytic stage of growth and development through soil. The sensitivity of mycelium and conidia to soil alone, and in residue, indicate that the fungus persists in nonliving host material that previously was parasitized. The organism persists for 18 mo as a saprophyte in tissue colonized during parasitism (9), but conidia or mycelia in the absence of residue lysed within a few days. Data presented here and elsewhere (4,5) show that many fungi that do not produce special resting structures; ie, sclerotia, chlamydo spores, oospores, etc. do not survive independently in soil for more than a few days.

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