Survival of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* in Rice Irrigation Water and Soil

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ABSTRACT

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Survival of spores and mycelium of *Colletotrichum gloeosporioides*, incitant of northern jointvetch anthracnose, was studied in host debris, rice irrigation water, and soil under laboratory and field conditions. The fungus was not recovered from soil collected from artificially infested field plots after 9 wk and was only occasionally reisolated from host debris buried in soil for more than 2 wk. Recovery was successful from host debris left above the soil on plants collected 7 mo after field inoculation. Under laboratory conditions, the fungus was recovered from sterile water but not from rice irrigation water 180 days after infestation with spores. Populations of *C. gloeosporioides* introduced into field soil stored in the laboratory declined to less than 1% of the original population within 4 wk but increased 8 to 80 times in the sterile soil control in the same time period. Sclerotia or sexual fruiting bodies of the fungus have not been found in culture or in infested plant debris.

Additional key words: biological control, mycoherbicide, pest management

The fungus *Colletotrichum gloeosporioides* (Penz) Sacc. f. sp. *aeschynomene* is the causal agent of anthracnose of northern jointvetch, *Aeschynomene virginica* (L.) B.S.P. Since 1970 this fungus disease has been used, on an experimental basis, as a biological control of this weed in Arkansas rice fields (3,10). The fungus is widely distributed in eastern Arkansas (14). Despite continued application of the fungus, the disease has not become an important mechanism to control the weed under natural conditions and annual applications of the fungus are necessary for control (11). Only slight increases in disease incidence have been recorded in fields treated with the fungus the previous season (*unpublished data*).

These observations suggest that the fungus does not overwinter well under prevalent field conditions or land management practices. Our research was conducted to identify the source of the primary inoculum and assess the overwintering capacity of the fungus. Survival of the fungus as spores or as mycelium in soil, rice irrigation water, and host plant debris was evaluated.

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MATERIALS AND METHODS

One isolate of *C. gloeosporioides*, maintained on Emerson's YpSs agar at 28 C, was used in all the experiments (4). Conidia were obtained by filtration of subcultures grown in modified Richard's V-8 juice medium (3). Spores in the filtrates were centrifuged and resuspended three times in sterile distilled water to remove excess media and were maintained free of contamination for laboratory experiments. No precautions were taken to maintain spores free of contamination when intended for field use.

Martin's medium (modified to include 0.2 g/L of streptomycin sulfate, 40 mg/L of chloramphenicol and 2.5 μg/L of nystatin) was used to isolate the fungus from soil, water, and plant debris in all experiments (12).

Survival of *C. gloeosporioides* in irrigation water. Ten liters of rice irrigation water was collected at random from each of two rice fields near Stuttgart, AR, in July 1976. Six 250-ml aliquots of the irrigation water were taken from each field sample and dispensed into six separate 500-ml Erlenmeyer flasks. Three flasks containing water from each field were stored at 10 and 28 C. Twelve 500-ml Erlenmeyer flasks, containing 250 ml of tap water, autoclaved twice at 24-hr intervals, served as controls for field water treatments at 10 and 28 C. All flasks were stoppered with cotton.

Spores of *C. gloeosporioides* used to inoculate the flasks were obtained under aseptic conditions and resuspended in sterile distilled water to a concentration of 1×10^6 spores per milliliter. All 24 flasks were inoculated with 0.25 ml of spore suspension to give a final calculated spore concentration of 1×10^3 spores per milliliter.

Flasks were periodically sampled to determine survival of the fungus. At each sampling, 0.1 ml of water from each flask was plated to each of five Martin's medium plates. After inoculated plates were incubated for 5 days at 28 C, the colonies of *C. gloeosporioides* were counted. Populations in the flasks were estimated from the average number of colonies on five agar plates.

Survival of *C. gloeosporioides* in soil. Field experiments were conducted on the Rice Branch Experiment Station, Stuttgart, AR, from November 1976 to March 1977 and from November 1977 to March 1978. Sixteen plots, 1 m^2, were laid out in randomized complete block design in four blocks of four plots each in fields under rice cultivation the previous summers. All plots were separated from adjacent plots by an alley 1 m wide.

Spores used to inoculate the soil were obtained from a 48-hr culture of *C. gloeosporioides* grown at 28 C in a 14-L Micro-Ferm fermentor (New Brunswick Scientific Co., Inc., New Brunswick, NJ). Spores were separated from mycelium by passage through a 250-mesh vibrating Sweco screen (Sweco, Inc., Los Angeles, CA), concentrated by low-speed centrifugation, and resuspended to final concentrations of 0.5×10^6, 2.0×10^6, and 8.0×10^6 spores per milliliter. One liter of inoculum of each spore concentration was applied as a drench to one plot in each block. Water was applied to the remaining plot in each block to serve as controls.

Populations of *C. gloeosporioides* were estimated periodically using Martin's medium in accordance with the following procedure. Five 1-g samples of soil, from the uppermost 2 cm, were taken from the center and from each quadrant of each plot, pooled, and placed in capped glass containers and kept at 4 C while in transit. Two subsamples from each pooled sample of soil, 0.5–1.0 g wet weight, were resuspended in 50 ml of water with a Virtis blender for 30 sec. After mixing, 0.1 ml of the soil suspensions was plated on each of five Martin's medium plates. The inoculated plates were incubated 5 days at 28 C before colonies of *C. gloeosporioides* were counted. The number of colonies of *C. gloeosporioides* per gram of wet soil was determined by averaging the two

subsamples. The number of colonies of *C. gloeosporioides* was adjusted to soil dry weight based on moisture content of soil at oven dry weight (110 C, 24 hr). Water content of soil ranged from 8 to 34%. The experiment was terminated in February 1977 and December 1977 after *C. gloeosporioides* was not reisolated from treated plots for two consecutive samplings.

Survival in soil in the laboratory. Soil (Crowley silt loam) was collected at the Rice Branch Experiment Station, air dried in the laboratory, and passed through a 4-mesh screen. One-hundred grams of screened soil was added to 20 250-ml Erlenmeyer flasks plugged with cotton. Ten flasks of soil were twice sterilized at 18 psi for 30 min. Ten milliliters of a spore suspension of *C. gloeosporioides*, aseptically collected and resuspended to a final concentration of 1×10^6 spores per milliliter, was added to each flask for a final calculated concentration of 100,000 spores per gram of soil. Treatments consisted of five replicates (flasks) of field soil or sterilized soil stored at 10 or 28 C. Populations of *C. gloeosporioides* were estimated on Martin's medium by soil dilution as described. The number of colonies of *C. gloeosporioides* was adjusted to soil dry weight based on moisture content of soil at oven dry weight (110 C, 24 hr).

All flasks were maintained at 15–20% moisture by addition of sterile water as needed and were stored in polyethylene bags.

Survival of *C. gloeosporioides* in plant debris. Two-hundred 6- to 8-wk-old seedlings (20 cm tall) of *A. virginica* were inoculated with a spore suspension of *C. gloeosporioides* containing 1×10^6 spores per milliliter. Plants were sprayed until runoff with an aerosol applicator. After inoculation, the plants were incubated in a dew chamber at 28 C for 24 hr, then returned to the greenhouse. After 1 wk, stems with visible lesions were excised from 80 plants and separated into eight groups of 10 stems each; each group was placed in a plastic bag, and the bags were stored at 4 C overnight before transport to the Rice Branch Experiment Station. The stems were mixed with 4–8 kg of soil, placed in a cotton gauze bag, and buried 10 cm beneath the soil surface 1 m apart in adjacent sites at the station in November of 1976 and 1977.

A single group of stems was recovered at random from the soil periodically for isolation of the fungus. Individual stems were separated from excess soil, washed in cold running tap water for 1–2 hr, immersed in 0.05% NaOCl for 30 sec and cut into 10 pieces 0.5 cm long. After these pieces were placed on Martin's medium and incubated 5 days at 28 C, examinations were made to determine percent recovery of the fungus from the 100 pieces. These experiments were terminated in March of 1976 and 1977.

In addition to the stems that were buried, three groups of 30 stems were placed in nylon mesh bags and suspended 1 m above the soil in November 1977. Ten stems were periodically recovered at random from the bags until March 1978. After a 1-cm section of each stem was removed, remaining portions of the stems were surface sterilized with 0.05% NaOCl and cut into 0.5-cm pieces that were placed on Martin's medium and incubated as described. The 1-cm sections removed earlier were placed in 1 ml of sterile water for 0.5 hr, after which the water was streaked to two plates to determine viability of spores on lesion surfaces.

RESULTS

Survival of *C. gloeosporioides* in irrigation water. *C. gloeosporioides* survived in sterile water and rice irrigation water incubated at 10 and 28 C for 120 days but not in irrigation water incubated at 10 or 28 C for 180 days (Table 1). Survival, expressed as colonies per milliliter of water, was significantly better in sterile water at 28 C than in sterile water at 10 C or in irrigation water at 10 or 28 C. The fungus population remained relatively stable until 150 days after inoculation in sterile water at 28 C, after which time it declined. There were no significant differences in survival among the other three treatments from 10 until 180 days after inoculation.

Survival of *C. gloeosporioides* in soil. Survival of *C. gloeosporioides* in soil in laboratory tests appeared to depend on storage temperature and soil sterility before artificial infestation (Table 2). *C. gloeosporioides* was recovered from sterile soil and field soil incubated at 10 and 28 C 6 wk after infestation. There were no significant differences in the number of colonies isolated per gram of dry soil among treatments, although recovery from sterile soil was many times greater than recovery from field soil. Lack of significance results from the variability in acquired data in all treatments. *C. gloeosporioides* was not reisolated from any of the samples taken from field soils left unsterilized 9 wk after inoculation, whether stored at 10 or 28 C.

Initial recovery of *C. gloeosporioides* from soil from field plots in 1976 was generally quantitatively dependent on the concentration of spores applied in drenches to the plots (Table 3). However, 2 wk after inoculation and thereafter, estimated populations of *C. gloeosporioides* in all plots had decreased to less than 5% of the initial population estimates and effects of inoculum concentrations were no longer evident or significant. After 4 wk, significantly greater estimates of *C. gloeosporioides* were obtained with soil of plots inoculated with 8×10^6 spores per milliliter. *C. gloeosporioides* was not recovered from soil of plots inoculated with 0.5×10^6 spores per milliliter 6, 9, and 12 wk after inoculation or from soil of plots inoculated with 2×10^6 or 8×10^6 spores per milliliter 9 and 12 wk after inoculation. The fungus was not isolated from soil of control plots at any time in 1976.

Data collected from the 1977 experiment (a repeat of 1976) differs markedly from data collected in 1976 (Table 3). For example, in 1977, *C. gloeosporioides* was reisolated from control plots. This may have resulted from contamination of control plots by spores from drenches applied to treated plots because heavy

Table 1. Survival of *Colletotrichum gloeosporioides* in sterile water and rice irrigation water during incubation in the laboratory at 10 or 28 C

Water	Incubation temp (C)	Colonies of *C. gloeosporioides* per milliliter of water[a] at day									
		0	10	20	30	45	60	90	120	150	180
Sterile	10	348 a	174 a	84 b	139 ab	72 a	59 b	67 ab	36 b	11 b	4 a
	28	305 a	216 a	219 a	329 a	132 a	146 a	129 a	160 a	147 a	60 a
Field	10	36 b	89 a	25 b	31 b	38 a	7 b	6 b	3 b	1 b	0 a
	28	55 b	17 a	8 b	20 b	4 a	1 b	1 b	2 b	0 b	0 a

[a] Data are the average of two experiments, three replicates of each treatment per experiment. All values in a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's new multiple range test (14).

Table 2. Survival of *Colletotrichum gloeosporioides* in field soil and sterilized field soil after artificial infestation and incubation at 10 or 28 C

Soil	Temp (C)	Colonies of *C. gloeosporioides* per gram of soil after incubation for the number of weeks indicated[a]				
		0.4	2	4	6	9
Sterile	10	6,648 b	13,526 b	84,175 b	160,338 b	7,719 a
	28	194,256 a	486,180 a	869,241 a	604,837 a	67,790 a
Field	10	3,305 b	1,006 c	582 c	329 a	0 a
	28	1,296 b	3,310 c	247 c	410 a	0 a

[a] All data are the average of five plates per replicate, five replicates each soil temperature treatment. All values in a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's new multiple range test (14).

Table 3. Survival of *Colletotrichum gloeosporioides* in soil of small field plots[a] artificially infested with various spore concentrations

Year	Spore concentration ($\times 10^6$ spores/ml)	Colonies of *C. gloeosporioides* per gram of (dry weight) soil[b] at week 0	2	4	6	9	12
1976	0	0 a	0 a	0 a	0 a	0	0
	0.5	1,402 a	248 a	55 a	0 a	0	0
	2.0	11,327 a	605 a	314 a	31 a	0	0
	8.0	57,100 b	311 a	2,115 b	23 a	0	0
1977	0	11,765	0	0			
	0.5	14,380	0	0			
	2.0	35,230	0	0			
	8.0	55,747	0	0			

[a] At the Rice Branch Experiment Station, Stuttgart, AR.
[b] Data are the average of two samples per replicate, four replicates per treatment. All values in a column from a given year followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's new multiple range test (14).

Table 4. Survival of *Colletotrichum gloeosporioides* in infested stems of *Aeschynomene virginica* buried in soil under field conditions in 1976 and 1977

Week buried	Sections (%) from which *C. gloeosporioides* reisolated 1976	1977
0	100[a]	75
2	20	27
4	1	1
6	0	7
9	0	0
12	4	...
15	0	0
18	...	0

[a] Data are the percent of 100 stem sections from which *C. gloeosporioides* was reisolated. At each interval, 10 stem segments excised from each of 10 infected stems were placed on nutrient agar plates.

rains before and during the experiment resulted in flooding of the field in which the experiment was conducted. In addition, *C. gloeosporioides* was not recovered at any time after the initial estimates at 0 wk. This rapid decline in the population may have also been caused by excessive soil moisture.

Survival of *C. gloeosporioides* in plant debris. Recovery of *C. gloeosporioides* from stem pieces buried in field soil became increasingly difficult with time. Although *C. gloeosporioides* was recovered from more than 75% of stem pieces buried overnight in the soil, within 2 wk after burial the fungus was recovered from 20 and 29% of the stem pieces in 1976 and 1977, respectively (Table 4). The fungus was only intermittently reisolated for buried stem pieces thereafter and was not recovered at all 9, 15, and 18 wk after inoculation in 1976 or 1977. The longest time at which *C. gloeosporioides* was reisolated from buried plant stems was just 12 wk. Reisolation seemed to depend on the degree of disintegration of the stems; *C. gloeosporioides* was rarely reisolated from disintegrated (rotting) stems. In both years, the experiment was terminated when land preparation for the next season began.

Because *C. gloeosporioides* was rapidly lost from buried stems, lesions were collected in March 1976 from infected plants left standing through the winter. Individual lesions were excised from stems and incubated 5 days at 28 C in petri dishes containing moistened filter paper. Although new sporulation on lesion surfaces was not observed after incubation in the chambers, the fungus was recovered from 24.7% of the 89 lesions when the lesions were immersed in sterile water blanks and shaken occasionally for 2 hr, after which time the water was streaked into Martin's medium with a platinum loop. This data indicated that *C. gloeosporioides* survived well in host refuse left above the soil, and more elaborate studies were conducted.

C. gloeosporioides was isolated from infected plant debris left above the soil surface as long as 18 wk after the experiment began (Table 4). Reisolation from aerial debris is in direct contrast to reisolation from tissue buried in soil. Recovery of *C. gloeosporioides* by direct isolation declined gradually from 100 to 14% by 18 wk. In contrast, recovery from washes was constant at 100% until 4 wk, declined to 90% until 9 wk, and dropped sharply thereafter to 0% at 18 wk. Decline in the recovery of *C. gloeosporioides* correlated with a gradual increase in the appearance of other fungi.

DISCUSSION

Evidence presented here suggests that *C. gloeosporioides* f. sp. *aeschynomene* is not a successful soil-inhabiting fungus as described by Menzies (7) because populations of the fungus in soil decline rapidly in the field and laboratory and because the fungus is rapidly lost from infected host stems placed in soil. Unlike *C. coccodes*, which produces sclerotia and can survive in soil for at least 1 yr (5), survival of *C. gloeosporioides* in soil as sclerotia is not likely since sclerotia are not produced in culture or within infected plants (9). Although Farley (5) found that *C. coccodes* persists in soil inoculated with spores for 1 yr, Blakeman and Hornby (1) previously reported that *C. coccodes* survives only 3 wk in soil in glasshouse experiments. Although the methods differed, both tests indicate that spores are not a significant survival propagule for *C. coccodes* and corroborate data for *C. gloeosporioides*.

The results also suggest that *C. gloeosporioides* overwinters most successfully on and in lesions of infected plants left standing over winter. Carroll et al (2) and Lukezic (6) previously showed that *C. trifolii* is also more easily recovered from stem sections left above the soil surface than from leaf tissue under crop residue. Survival of *C. trifolii* was more reliably determined by direct observation of new sporulation after incubation in moist chambers than by direct isolation because of contamination problems encountered in direct plating of tissue segments onto media. *C. gloeosporioides* was more reliably isolated from sterile water washes of lesions than by direct plating of tissue or by direct observation of new sporulation on lesions until 15 wk after the first exposure of the lesions.

Overwintering of the fungus in the field may depend on cultural practices since standing plant debris appears to be the source of primary inoculum for northern jointvetch anthracnose. Practices that bury or otherwise destroy lesions in the fall would reduce the level of primary inoculum. Spring cultivation of rice stubble would allow higher levels of primary inoculum to survive. However, an additional source of inoculum may be infested seed. Several *Colletotrichum* species, including *C. gloeosporioides* f. sp. *aeschynomene*, survive in or on the seeds of their hosts (8,13,15–17). Infestation and infection of seed of *A. virginica* resulted in a significant level of lethal seedling disease caused by *C. gloeosporioides* (8).

Low overwintering potential and a poor dispersal mechanism are major constraints on the buildup of this disease to epidemic levels under natural conditions. *C. gloeosporioides* cannot be expected to control the weed with the consistency required by agriculture without annual augmentation of the primary inoculum. Therefore, annual application of *C. gloeosporioides* would be required to effectively reduce infestations of this weed to levels below economic thresholds in Arkansas rice or soybean fields.

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