Effects of Inoculum Burial, Temperature, and Soil Moisture on Survival of *Cylindrocladium crotalariae* Microsclerotia in North Carolina

J. K. PATAKY, Graduate Research Assistant, and M. K. BEUTE, Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27650

ABSTRACT

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Survival of *Cylindrocladium crotalariae* microsclerotia on the soil surface during the winter was reduced compared with that of microsclerotia buried 25 cm below the soil surface. Less than 5% of the initial number of microsclerotia placed on the soil surface in November were recovered after 12 wk. Percentage recovery of initial inoculum from samples buried at 25 cm for 24 wk was 31, 2.2, and 55% for microsclerotia on stems in 1981, microsclerotia on stems in 1982, and microsclerotia in soil in 1982, respectively. Moisture content and weight of buried samples were less variable than for samples placed on the soil surface. Viability of microsclerotia at 4 C was greater in very moist soil than in dry soil. Temperature and moisture appeared to interact in their effect on microsclerotial viability, which may partially explain the increased ability of microsclerotia to survive when buried.

Additional key words: *Arachis hypogaea*, Cylindrocladium black rot (CBR)

Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea* L.) is a peg, pod, and root rot caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers (1). Microsclerotia are the survival structures and primary inocula of *C. crotalariae*, a soilborne pathogen. Large numbers of microsclerotia are formed in the cortex of infected peanut roots, in peanut root nodules, and in roots of other leguminous hosts (6,17).

Populations of microsclerotia in naturally infested peanut soils generally ranged from 0 to 100 microsclerotia per gram of soil in the early 1970s, when CBR was first reported in North Carolina and Virginia (8,12,14). In recent years, microsclerotial populations have commonly ranged from 0 to 20 microsclerotia per gram of field soil (5,11,18). Spatial distributions of microsclerotia in naturally infested soil are contagious (5,18), which is probably the result of spatial distributions of diseased plants in which microsclerotia form and are released when roots decay.

Currently, no single practice is adequate to control CBR so several practices are integrated into a CBR management program. Effectiveness of CBR management tactics is dependent on inoculum densities of *C. crotalariae*. For example, resistance to CBR is inoculum density-dependent (4,9), with resistant genotypes becoming severely diseased at high microsclerotial populations. Effectiveness of crop rotations is also inoculum density-dependent. Rotations to nonhosts reduced the percentage of soil samples from which *C. crotalariae* was recovered (16), but 2-yr rotations did not consistently reduce inoculum densities to levels at which susceptible commercial cultivars could have been grown economically (10). Rotations to other leguminous hosts increased inoculum density (7,10).

Inoculum density at planting may determine the success or failure of a peanut crop. Preliminary evaluations of CBR forecasts based on estimates of inoculum density before planting have been made (11). At present, the best CBR management strategy appears to be reduction of inoculum density and use of a partially resistant cultivar.

Several researchers have reported that inoculum density is reduced at low temperatures (2,3,10,15,18). Freezing or desiccation caused by freezing appear to greatly decrease microsclerotial viability. Griffin et al (2) have also reported that recovery of microsclerotia from soil samples is reduced when soil is air-dried, but rewetting of soils partially negates the effect of drying. Rowe et al (17) reported the survival of microsclerotia in air-dried roots.

Peanut roots and root fragments are usually scattered on the soil surface at harvest. Rowe et al (17) incorporated *C. crotalariae*-infected root fragments into soil in December and demonstrated that peanuts planted in the soil the following spring were infected. Phipps and Garren (13) indicated that overwintering of debris on the soil surface will decrease microsclerotial survival. The objectives of this study were to compare the overwinter survival of *C. crotalariae* microsclerotia buried in soil with that of microsclerotia on the soil surface and to evaluate the effect of soil temperature and moisture on the viability of microsclerotia.

MATERIALS AND METHODS

Field experiment designs. Survival of microsclerotia below and on the soil surface was studied during the winter of 1980–1981 at Clayton, NC, and the winter of 1981–1982 at Raleigh, NC. In the 1980–1981 study, stem pieces 10 cm long containing microsclerotia were either buried about 25 cm below the soil surface or placed on the soil surface. Samples were collected on seven dates at 4-wk intervals from 19 November 1980 to 5 May 1981. Treatments were arranged in a completely randomized design (with respect to sampling dates) with five replicates. In the 1981–1982 trial, treatments included buried and soil surface samples of both microsclerotia on peanut stems and microsclerotia mixed in peanut field soil. In 1981–1982, treatments were arranged in a completely randomized design with three replicates. Sampling dates were from 23 November 1981 to 10 May 1982.

Microsclerotia production. Microsclerotia were produced on peanut stems and in potato-dextrose agar (PDA) cultures. Peanut stems 10 cm long and of similar diameter were sterilized in 250-ml flasks, 50 stems per flask. Ten milliliters of sterile water and a 0.5-cm-diameter disk from each of 10 *C. crotalariae* isolates cultured on PDA were added to each flask. Microsclerotia were abundant on stems after 6 wk. One stem was taken from each of 10 flasks. The 10 stems were then weighed and placed in a nylon mesh bag, which was then sealed. A total of 70 and 42 bags of 10 stems each were prepared for 1980–1981 and 1981–1982, respectively.

Isolates of *C. crotalariae* were also cultured on PDA to produce microsclerotia for overwintering tests. Microsclerotia were abundant on PDA after 4 wk. Cultures were comminuted in a blender for 3 min (1.5 min at slow speed, 1.5 min at high speed) and washed through a 270-mesh (50 μm) sieve. Microsclerotia collected on the sieve were added to 2,500 g of peanut field soil collected from a field

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Present address of the first author: Department of Plant Pathology, University of Illinois, Urbana 61801.

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with no previous history of CBR. Infested soil was placed in a polyethylene bag and mixed thoroughly by shaking by hand in the bag for 5 min. Fifty grams of infested soil was placed in each nylon mesh bag, which was then sealed. Forty-two bags were prepared.

Assay of microsclerotia. To assay viability (ability to produce colonies) of microsclerotia on peanut stems, stems were removed from bags, weighed, and cut into small pieces over a 400-mesh (38 μm) sieve. The pieces were blended for 3 min (1.5 min at low speed, 1.5 min at high speed), rinsed on a 400-mesh sieve, washed in 0.25% sodium hypochlorite for 1 min, rinsed on a 400-mesh sieve, and suspended in 200 ml of water. Five and 10 ml of the suspension were assayed on a semiselective medium as described by Phipps et al (12). The suspension was further diluted with water to 770 ml and 5- and 10-ml samples were again assayed. The number of viable microsclerotia was determined at the dilutions at which colonies of *C. crotalariae* could be adequately counted. The assay procedure for microsclerotia in soil was the same as that for stems except for the cutting and blending of stems.

Soil moisture study. The effect of soil moisture on microsclerotial viability was studied at 4 and 16 C in incubation chambers (Lab-line Ambi-hi-lo chamber, Lab-line Instruments, Inc., Melrose Park, IL). Microsclerotia were produced on PDA and prepared for infestation of peanut field soil as described previously.

Treatments included four matric potentials of about −0.1, −0.3, −1, and −5 bars. Matric potentials were equivalent to 12.7, 9.3, 6.9, and 3.8% water (w/w), based on data from soil moisture release curves for the peanut field soil. To achieve these moistures, appropriate amounts of air-dried soil were mixed with 10 g less than the appropriate amounts of water to give a total weight of 1,200 g. Soil and water were stored in polyethylene bags for 3 days before adding 10 ml of a microsclerotial suspension. The inoculum was thoroughly mixed by shaking by hand for 5 min.

Samples of 110 g were placed into 100-ml screwcap vials and incubated at 4 and 16 C. Four replicates of four treatments were arranged in a completely randomized design in both chambers. Soil moistures were monitored weekly by weighing samples in vials, and if necessary, water was added to the vials. The soil was sampled seven times at 4-wk intervals and assayed for microsclerotia. On each sampling date 11.3, 10.9, 10.7, and 10.4 g (10 g soil on a dry-weight basis) were assayed from vials containing soil at −0.1, −0.3, −1, and −5 bars, respectively.

After 20 wk, soil from each vial in the 4 C chamber was divided into three subsamples. One subsample was returned to 4 C for 4 wk. One subsample was maintained at the same matric potential but moved to the 16 C chamber for 4 wk. One subsample was flooded with 2 ml of water and moved to the 16 C chamber for 4 wk. Similarly, soil in vials in the 16 C chamber were divided into two subsamples. One subsample was unchanged and the other was flooded and returned to the 16 C chamber for 4 wk.

RESULTS

Field experiment. Survival of microsclerotia on the soil surface was reduced compared with microsclerotia buried 25 cm below the soil surface (Table 1). Less than 1% of the initial number of microsclerotia cultured on peanut stems and less than 7% of the microsclerotia added to peanut field soil were recovered after 8 wk on the soil surface. In contrast, percentage recovery of buried microsclerotia after 24 wk was 31, 2.2, and 55 for microsclerotia on stems in 1981, microsclerotia on stems in 1982, and microsclerotia in soil in 1982, respectively.

Position, time (duration of exposure), and the position × time interaction term were significant in analyses of variance ($P < 0.05$), indicating that both time and position affected microsclerotial survival, but the response over time differed by position. Therefore, regressions of $\log_{10}$ (microsclerotia + 1) on time were different for the buried microsclerotia than for microsclerotia on the soil surface (Fig. 1). Similarly, the medium (stem tissue or soil) × time interaction term was significant for the 1981–1982 trial, which indicates that regressions for microsclerotia cultured on stems and those added to soil were different (Fig. 1B,C).

Differences in moisture content were observed for buried and surface samples. Buried samples were moist at all sampling dates. Buried samples also weighed more at all sampling dates than at the beginning of the study. For samples on the soil surface, weight and moisture content varied among sampling dates and were probably affected by rainfall.

In the first 16 wk of the trials, mean daily air temperature was below 6 C for 68 and 65 days and below 0 C for 20 and 15 days in 1980–1981 and 1981–1982, respectively. During the final 8 wk of the trials, temperature was below 6 C only 7 and 5 days in 1981 and 1982, respectively.

Recovery of viable microsclerotia increased slightly in the spring of both years. Percentage recovery of microsclerotia was lowest at 16 wk (9 and 11 March) for all treatments except microsclerotia in soil on the soil surface in 1982 (Table 1). Regressions for microsclerotia cultured on stems also indicated that increased recovery of microsclerotia in the spring was statistically significant because the quadratic terms were significant and inflection points of the quadratic equations were from 15 to 21 wk (Fig. 1A,B). These increases correspond to warmer temperatures in March through May.

Soil moisture study. The effect of soil moisture on microsclerotial viability differed in trials conducted in the 4 and 16 C chambers. At 16 C, soil moisture had no effect on microsclerotial viability. Mean initial inoculum density was about 35 microsclerotia per gram of soil. Throughout the study, more than 90% of the microsclerotia were viable from all samples at 16 C. Flooding samples for 4 wk also had no effect on microsclerotial viability for samples incubated at 16 C because more than 90% of the microsclerotia from flooded samples were viable.

At 4 C, microsclerotial viability was highest in moist soil (Table 2). After 16 wk, no microsclerotia were recovered from samples at −5, −1, and −0.3 bars; however, 19% of the microsclerotia were viable from samples at −0.1 bar.

When samples incubated at 4 C for 20 wk were moved to 16 C or flooded and moved to 16 C for 4 wk, no microsclerotia were viable from samples initially at −5 and −1 bars. However, for samples initially at −0.1 bar, 15% of the microsclerotia were viable in the nonflooded and 22% of the microsclerotia were viable in the flooded treatments that were moved to 16 C. Only 1% of the microsclerotia held at 4 C for 24 wk were viable.

Table 1. Percentage recovery of viable *Cylindrocladium crotalariae* microsclerotia on peanut stems or in peanut field soil on the soil surface or buried 25 cm below the soil surface

		Percentage recovery of viable microsclerotia[a]					
		1980–1981[b]		1981–1982[c]			
		Stems		Stems		Soil	
Period	Week	Surface	Buried	Surface	Buried	Surface	Buried
Nov.	0	100.0	100	100.0	100.0	100.0	100
Nov.–Dec.	4	16.0	94	2.2	46.0	84.0	100
Dec.–Jan.	8	0.4	60	<0.01	1.3	6.5	61
Jan.–Feb.	12	0.4	21	0.4	1.6	4.9	97
Feb.–Mar.	16	0.04	14	<0.01	0.3	2.0	52
Mar.–Apr.	20	0.1	44	0.04	1.2	0.8	73
Apr.–May	24	0.04	31	0.02	2.2	0.5	55

[a] Percentage recovery of viable microsclerotia determined as percentage of number of microsclerotia that germinated and produced colonies at week 0 (November).
[b] 1980–1981 Trials conducted at Central Crops Research Station, Clayton, NC.
[c] 1981–1982 Trials conducted in Raleigh, NC.

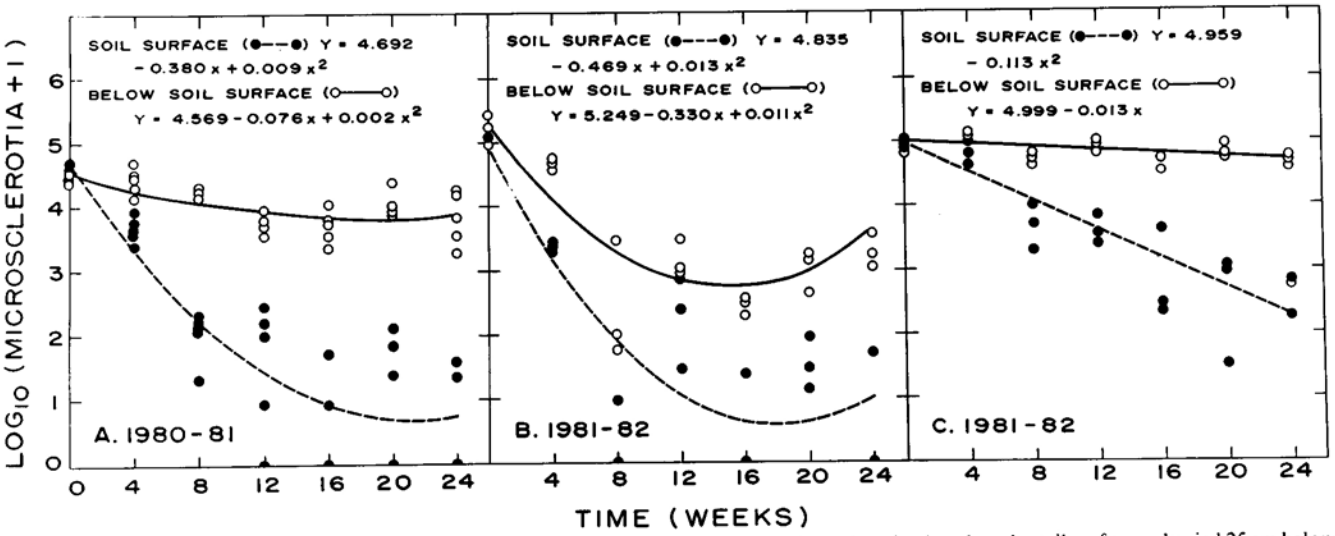

Fig. 1. Regressions of $\log_{10}$ (microsclerotia + 1) on time for *Cylindrocladium crotalariae* microsclerotia placed on the soil surface or buried 25 cm below the soil surface: **(A)** microsclerotia cultured on peanut stems and overwintered at Clayton, NC, 1980–1981, **(B)** microsclerotia cultured on stems and overwintered at Raleigh, NC, 1981–1982, and **(C)** microsclerotia added to peanut field soil and overwintered at Raleigh, NC, 1981–1982. Samples for which no microsclerotia were viable are represented as half-circles (eg, soil surface samples at 12, 16, 20, and 24 wk in 1980–1981).

DISCUSSION

Our results indicate that microsclerotia of *C. crotalariae* dispersed on the soil surface during peanut harvest are less likely to survive than are those that remain buried at harvest or those buried by fall tillage operations. Microsclerotia can be dispersed on the soil surface in root fragments during harvest (17) but we know of no published data that compare vertical distributions of microsclerotia in the soil profile after harvest. If most microsclerotia are on the soil surface, fall tillage should be avoided in fields in which CBR is the primary disease problem.

Temperature and moisture appear to interact in their effects on microsclerotial viability. Our results indicate that prolonged exposure to low temperatures decreases microsclerotial viability. These results agree with those of others (2,3,10,15). When soil matric potential was at or below −0.3 bars, fewer microsclerotia were viable at low temperatures than when matric potential was above −0.3 bars. This interaction of temperature and moisture may partially explain the ability of buried microsclerotia to survive better than microsclerotia on the soil surface. At 16 C, soil moisture had no effect on microsclerotial viability over the range of matric potentials evaluated in this study. Griffin et al (2) reported decreased microsclerotial germinability in very dry soil (about −2,000 bars).

The ability of microsclerotia to recover from the effects of low temperatures appears to be increased by moisture. For samples incubated at 4 C for 20 wk, only those at −0.1 or −0.3 bars responded to subsequent incubation at 16 C for 4 wk. Recovery of viable microsclerotia increased with temperature and agrees with results of others (3,15) and with the results from our field trials in which percentage recovery of initial inoculum on peanut stems was greater in April and May than in March. These increases could have resulted from increased germination of existing microsclerotia, fragmentation of existing microsclerotia to form smaller viable microsclerotia, or germination of existing microsclerotia to form secondary microsclerotia. Because of this phenomenon, soil samples collected in late winter or early spring should be stored at warmer temperatures before assaying for *C. crotalariae* microsclerotia in order to obtain more accurate estimates of inoculum density.

Currently, data are not sufficient to predict microsclerotial survival based on temperature, moisture, and/or time. Regression equations from our field trials were analytical rather than predictive. Results of our field trials indicate that after 16 wk, in which the mean daily air temperature was below 6 C for more than 65 days, percentage recovery of viable microsclerotia on the soil surface was less than 2% of the initial inoculum. Results from incubation studies indicate that microsclerotia were not viable after more than 16 wk at 4 C unless soil moisture was high. These results and those of others (2,10,15) indicate that further investigations of microsclerotial survival may produce useful CBR forecasts. In particular, information concerning vertical distributions of microsclerotia overwintering in peanut field soils, effects of temperatures between 0 and 16 C on microsclerotial survival, effects of fluctuating temperatures and moistures on microsclerotial survival, and effects of short durations of freezing temperatures may be useful in developing predictive models.

Table 2. Percentage recovery of viable *Cylindrocladium crotalariae* microsclerotia incubated at 4 C in soil at different matric potentials

Week	Percentage recovery of viable microsclerotia[a] (matric potential [bars])			
	−0.1	−0.3	−1	−5
0	100	100	100	100
4	95	92	100	80
8	36	12	17	13
12	41	2	1	4
16	19	0	0	0
20	2	0	0	0
24	1	0	0	0
20 + 4[b]	15	2	0	0
20 + 4 (F)[c]	22	0	0	0

[a] Percentage recovery of viable microsclerotia determined as percentage of the number of microsclerotia that germinated and produced colonies at week 0 (35 microsclerotia per gram of soil).
[b] 20 Wk at 4 C and 4 wk at 16 C.
[c] 20 Wk at 4 C and 4 wk at 16 C in flooded soil.

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