Population Dynamics of Cylindrocladium crotalariae Microsclerotia in Naturally-Infested Soil

P. M. Phipps and M. K. Beute

Visiting assistant professor and associate professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607.

Present address of senior author: Tidewater Research and Continuing Education Center, Holland Station, Suffolk, VA 23437.

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ABSTRACT

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Microplots of soil in 70-cm diameter fiberglass cylinders were used during a 2-yr period to study the dynamics of populations of *Cylindrocladium crotalariae* microsclerotia (MS) in a naturally-infested field. The effect of fallow, four rotational crops (corn, cotton, tobacco, and soybean), and three peanut cultivars on MS densities in soil was studied over two growing seasons. All three peanut cultivars and soybean were susceptible to infection by *C. crotalariae*, but corn, cotton, and tobacco were immune. Low rainfall in the first growing season limited disease development, and MS populations did not change significantly in soil planted to host crops. Slight but significant ($P = 0.05$) reductions in MS densities were detected in fallow soil and soil planted to nonhost crops. After harvest and incorporation of crop residues in soil, MS densities did not change significantly ($P = 0.05$), according to soil assays in January and April. Florigiant, a susceptible peanut cultivar, developed severe symptoms of Cylindrocladium black rot (CBR) in the second growing season. Soybean and the CBR-resistant peanut cultivars, Argentine and NC3033, showed few or no symptoms. Populations of MS at harvest were 9.6, 5.2, and 1.6 times preplant densities in soils planted to the peanut cultivars, Florigiant, Argentine and NC3033, respectively. MS densities increased 3.7 times in soils planted to soybean. As in the previous growing season, MS densities at harvest were slightly less than preplant densities in fallow soil and soil planted to nonhost crops. After harvest and incorporation of crop residues in soil, MS densities declined sharply between January and April, when soil water in the plow layer froze during several weeks of subnormal temperatures. Field and laboratory tests supported the conclusion that low soil temperatures (≤5 C) caused this sharp decline in MS populations.

Additional key words: Arachis hypogaea L., *Calonectria crotalariae*.

Cylindrocladium crotalariae (Loos) Bell and Sobers (1) causes a peg, pod, and root necrosis in peanuts (*Arachis hypogaea* L.) that is commonly referred to as Cylindrocladium black rot (CBR). Since the first report of CBR in North Carolina and Virginia (2), the disease has spread at an alarming rate in both states (11,17). *C. crotalariae* also causes disease in soybeans in North Carolina and Virginia (5,16). Greenhouse tests have indicated that tobacco and cotton are susceptible to root infection by *C. crotalariae* and that corn, wheat, and rye are resistant (15). *C. crotalariae* has not been reported to parasitize or cause disease in cotton or tobacco grown in naturally-infested soil in the field.

Microsclerotia (MS) of *C. crotalariae* form in the cortex of infected peanut roots and are the primary survival structure of this fungus in naturally-infested soil (4,10,18). Development of rapid, efficient procedures for quantitative estimation of MS populations in soil have provided technology for detailed studies on the epidemiology of CBR in peanuts (6,10).

The primary objectives of our study were to determine: i) the effects of peanut cultivars, rotational crops (ie, corn, cotton, soybean, and tobacco), and fallow on populations of *C. crotalariae* MS in naturally-infested soil in the field; and ii) the role of these crops in the epidemiology of CBR in peanuts.

MATERIALS AND METHODS

Field site and installation of microplots. Experiments were conducted in a naturally-infested peanut field (Norfolk sandy loam) near Roanoke Rapids in northeast North Carolina. Microplots (70-cm diameter) were constructed from fiberglass sheets (0.3 cm thick, 60 cm wide, 245 cm long) and inserted in soil, leaving 25 cm above ground as a splash barrier. Microplots were installed without disrupting the soil profile by making a 0.5–1 cm wide cut in soil with a circular cutter driven by a posthole digger on a tractor.

Soil sampling and assay. Vertical core samples (2 × 15 cm) were collected from each microplot with a soil sampling tube. Soil from each microplot was placed in polyethylene bags, closed with wire tags to prevent moisture loss, and stored at room temperature (23–27 C). Within 2 wk after collection, each soil sample was mixed thoroughly by hand, analyzed gravimetrically for moisture content, and assayed for *C. crotalariae* MS by the elutriation method (10).

Selection and culture of crops. One susceptible (Florigiant) and two resistant (Argentine, NC3033) peanut cultivars were selected (8). Tobacco (Speight G28), cotton (Coker 310), corn (Pioneer 3369A), and soybean (Ransom) were selected on the basis of current agronomic practices in North Carolina. On 19 May 1975, four replicate microplots were selected at random for planting to each crop and four more were selected for fallow. Granular 3-9-9 fertilizer (22.4 kg of N/ha, 67.2 kg of P_2O_5/ha, 67.2 kg of K_2O/ha) and aldicarb (19 kg of 15G formulation/ha) were incorporated in all microplots. No herbicides were applied for weed control. Tobacco was obtained from a transplant bed, but all other crops were established with seed. Plant densities were standardized at two plants per microplot for tobacco and three plants per microplot for all other crops. Foliar sprays of benomyl (0.56 kg of 50WP formulation/ha) were applied at 2-wk intervals after 1 July to peanuts for control of Cercospora leafspot. Carbaryl (1.12 kg of 80WP formulation/ha) was applied to all crops as needed to control certain insect pests. Landplaster (1,120 kg of $CaSO_4$/ha) was applied to the soil surface on 7 July in microplots planted to peanuts.

On 4 May 1976, each crop was planted in the same microplots as

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in 1975. In addition, 24 microplots were planted to each crop (except Argentine peanuts) in the same field. Soil in these microplots had been planted to Florigiant peanuts in 1975 and wheat during the winter of 1975–76. The same cultural and pest control practices were used in 1975 and 1976.

Harvesting of each mature crop simulated standard practices. After removing the fruit, the residues were cut into segments smaller than 10 cm and mixed into the top 15 cm of soil. Mature tobacco leaves were pulled during each growing season. In September, tobacco stalks and roots were cut and mixed into soil.

Disease incidence and root infection. During each growing season, disease incidence was monitored by recording aboveground symptoms and the occurrence of perithecia of *Calonectria crotalariae* (perfect stage of *C. crotalariae*) on diseased tissues. Root rot was evaluated in each crop at harvest on a scale of 0 (no visible damage) to 5 (completely destroyed) as reported previously (8). To confirm that root damage in each crop was caused by *C. crotalariae*, three roots per plant were collected, treated for 1 min in 0.5% NaClO, and assayed for *C. crotalariae* on the Tergitol (NPX) medium (10) amended with oxgall (4 g/L) and pentachloronitrobenzene (67 mg of 75% wettable powder formulation per liter) (6).

MS production by *C. crotalariae* in fibrous roots of crops was ascertained by collecting and examining roots from plants in each microplot according to the procedure described by Rowe et al (17). Five slides of roots (six 4.5-cm long roots per slide) from each microplot were prepared with lactophenol.

RESULTS

Dynamics of microsclerotia in soil. Populations in soil from microplots in April 1975 ranged from 14.5 to 24.1 MS per gram of soil (Fig. 1). In microplots planted to the peanut varieties or soybean, MS densities did not differ significantly ($P = 0.05$) in soil sampled in October 1975, January 1976, or April 1976 (Fig. 1). In fallow soil and in soil planted to tobacco, cotton, or corn, the reduction in MS densities was significant ($P = 0.05$) in soil sampled in October 1975. MS densities in soil did not decline further according to soil assays in January 1976 or April 1976. Comparison of MS densities in soil collected in April 1976 showed no significant differences, regardless of the cropping practice in 1975.

After harvest of crops in October 1976, marked increases ($P = 0.05$) in MS populations were found in soil planted to Florigiant (CBR-susceptible) and Argentine (CBR-resistant) peanuts and to soybean (Fig. 1). MS densities were 9.6 times greater than the preplant population in soil planted to Florigiant peanut and 5.2 and 3.7 times greater in soil planted to Argentine peanut and Ransom soybean, respectively. Preplant populations increased 1.5 times (not significant at $P = 0.05$) in soil planted to NC3033 peanut. In fallow soil and soil planted to tobacco, cotton, or corn, MS densities exhibited little change. MS populations decreased significantly ($P = 0.05$) only in microplots planted to corn. Although assays of soil collected 5 January 1977 indicated a decline in MS densities in soil from all microplots compared with densities in October, the decrease was significant ($P = 0.05$) only in the soil planted to Florigiant peanut. MS populations decreased markedly by April 1977, particularly in soils with high densities of MS in January 1977. Densities of MS in soil ranged from 0.6 to 1.9 MS per gram of soil in April 1977.

In the 20 additional microplots established in April 1976, the dynamics of MS densities were essentially the same as that reported above. In these microplots, MS populations increased significantly ($P = 0.05$) in soil planted to Florigiant peanut and Ransom soybean but not in soil planted to NC3033 peanut, cotton, tobacco, or corn. Fallow soil and Argentine peanut were not evaluated in this second test. Comparison of MS densities in plots corresponding to those planted to a given crop in the original test showed no significant differences ($P = 0.05$) at a given sampling time.

Disease incidence and root infection. At harvest in October 1975, neither CBR symptoms above ground nor perithecia of *Calonectria crotalariae* were observed. Only peanut and soybean roots exhibited any necrosis. Root tissue assays yielded *C. crotalariae* from the three peanut varieties and soybean but not from tobacco, cotton, or corn.

In September 1976, symptoms of CBR and perithecia of *C. crotalariae* were noted for at least one plant in each microplot planted to Florigiant peanut. No aboveground symptoms or signs of CBR were observed in plots planted to other crops, with the exception of one NC3033 peanut plant. Root rot was most severe in Florigiant peanut; soybean and the peanut cultivars NC3033 and Argentine appeared to have minimal root damage (Table 1). Root tissue biopsies indicated a lower degree of infection by *C.*

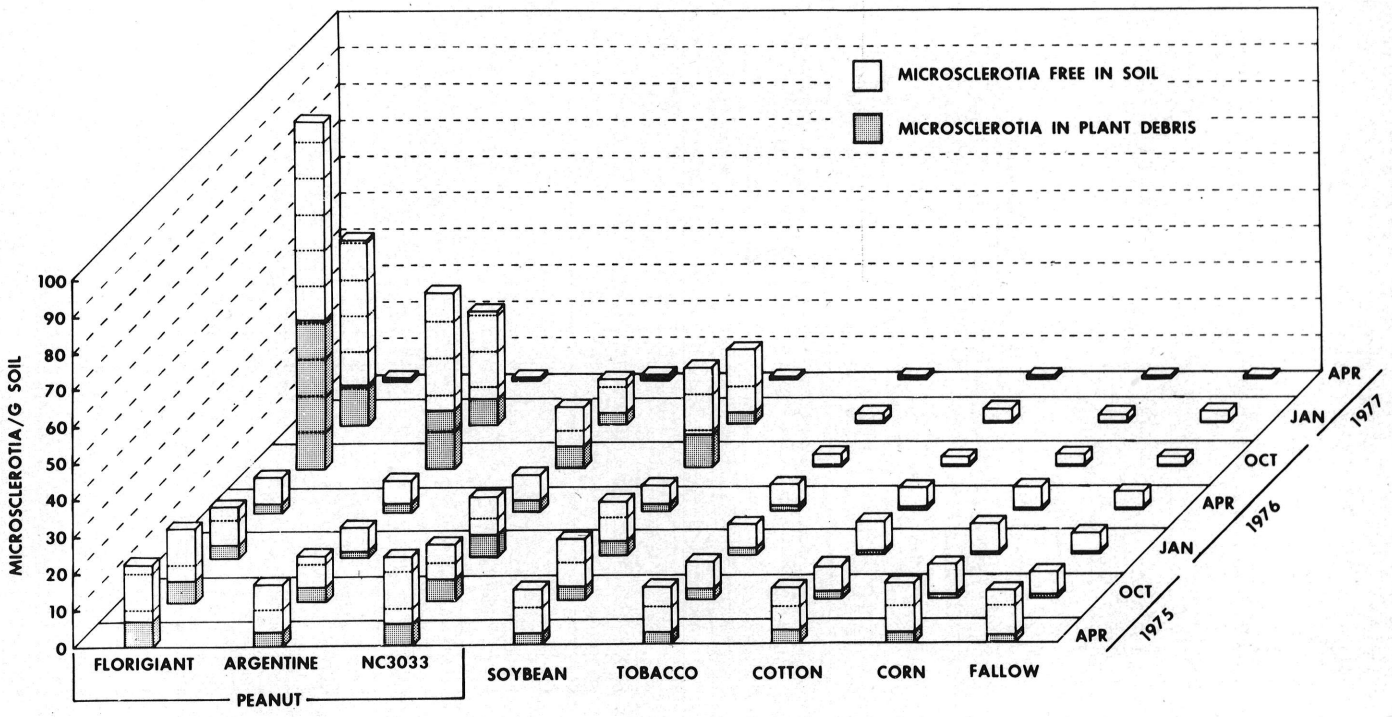

Fig. 1. Population dynamics of *Cylindrocladium crotalariae* microsclerotia in fallow soil and soil planted to three peanut cultivars, soybean, corn, cotton, and tobacco.

crotalariae in NC3033 peanut than in soybean, Florigiant peanut, or Argentine peanut. None of the 72 root biopsy tissues from tobacco, cotton, or corn yielded *C. crotalariae*.

Microsclerotia in cleared roots. Fibrous roots from Florigiant peanut contained high densities of MS in the cortex as shown by Rowe et al (18). Argentine peanut and Ransom soybean had a similar incidence of MS in fibrous roots, although densities were low compared with Florigiant peanut. All three hosts, however, contained high densities of MS in root nodules.

NC3033 peanut contained only a few MS per root and nodule specimen. In most instances, these MS were primarily on the outer surface of roots rather than in the cortex.

No MS were found in fibrous roots from tobacco, cotton, or corn.

Climatological data summary. Climatological records from a weather station near the field site were used to summarize rainfall (Fig. 2) and air temperatures (Fig. 3). In the 1975 growing season, rainfall did not exceed 20 mm per day until 11 July. After the heavy rainfall between 8 and 18 July, drought extended until 1 September. In contrast to 1975, in the 1976 growing season, rainfall was more evenly distributed, with no prolonged drought. Daily maximum and minimum air temperatures during the 1975-76 winter were never at or below 0 C for long enough to freeze soil water, except at the surface. During January 1977, air temperatures were below 0 C for several consecutive days. Personal communication with farmers and our observations indicated that soil water froze in the plow layer (0–15 cm depth) for several weeks in January and February 1977.

TABLE 1. Incidence and severity of Cylindrocladium black rot in peanut cultivars and soybean grown in soil naturally-infested with *Cylindrocladium crotalariae* in 1976

Crop and cultivar	Plants with signs and symptoms[x] (%)	Root rot severity index (0–5)[y]	Recovery of *C. crotalariae* from root tissues[z] (%)
Peanut			
Florigiant	67	3.0 a	92
Argentine	0	0.8 b	56
NC3033	4	0.6 b	39
Soybean			
Ransom	0	0.5 b	61

[x] Each crop consisted of 12 plants.
[y] Severity index is 0 (no visible damage) to 5 (completely destroyed). Means (average of four replicates) for disease severity indices in columns followed by same letter(s) are not significantly different at $P = 0.05$ according to Duncan's new multiple range test.
[z] Percent of 36 root tissues (three per plant) yielding *C. crotalariae* in culture.

Effects of soil temperature. In laboratory experiments with naturally-infested soil from the location of microplots, soil temperature was an important factor affecting survival of MS. Storage of naturally-infested soil at room temperature (23–27 C) in polyethylene bags to maintain 5% moisture (w/w) resulted in no significant change ($P = 0.05$) in MS populations according to assays after 1 or 5-wk incubation. A 27 and 60% reduction in MS populations ($P = 0.05$) resulted in soil incubated at 5 C (+4 C) for 1 and 5 wk incubation, respectively.

In an additional experiment, naturally-infested soil was incubated in a greenhouse (20–28 C) and in a field near Raleigh, NC, from November 1976 to April 1977. At each location, four replicate lots of soil were placed in wooden flats. Sod and soil were packed around the sides of each flat in the field, and at both locations soils were kept moist. In November, MS populations in the eight lots of soil averaged 117.3 MS per gram of soil (range 92.7–158.5). Assays of soil samples collected in April 1977 yielded 0–2.7 MS per gram of soil incubated in field conditions. MS densities did not change significantly ($P = 0.05$) in soil incubated in the greenhouse. Mean temperatures were less than 0 C for 14 days in January and 3 days in February 1977 according to measurements at a 10-cm depth in a fallow, Granville sandy loam near the field site.

DISCUSSION

Previous reports (4,18) and our observations indicate that MS of *C. crotalariae* are formed after infection and colonization of host plant roots. In addition to formation in the cortex, large numbers of MS also may develop in root nodules of leguminous hosts, such as peanut and soybean. Although *C. crotalariae* can invade leaf and stem tissues of several plants (13), current evidence indicates that, in North Carolina peanut production areas, MS develop only in living roots of host crops such as peanuts or soybean. Further-

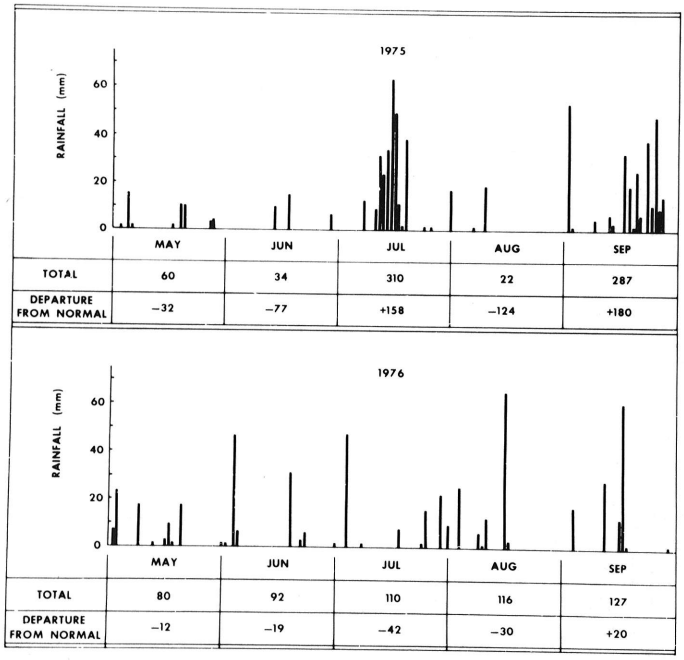

Fig. 2. Amounts and occurrence of rainfall during the 1975 and 1976 growing seasons at Roanoke Rapids, NC.

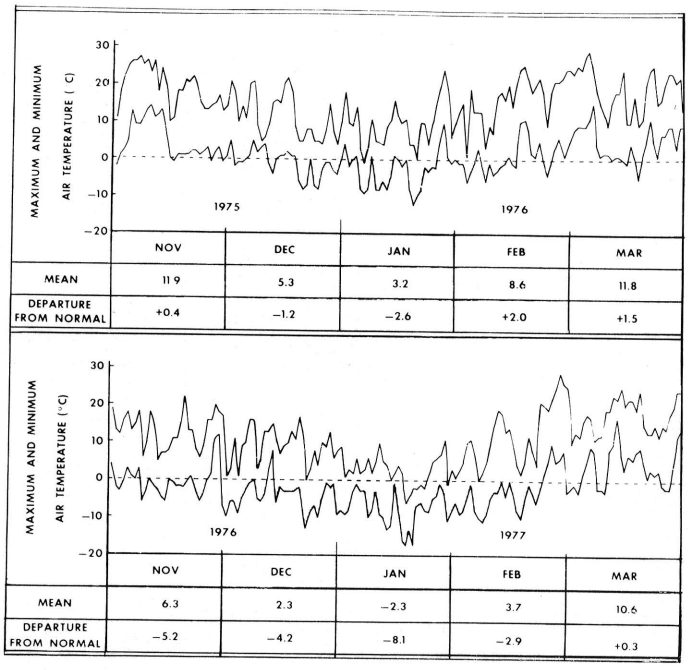

Fig. 3. Maximum and minimum air temperatures during the winter months of 1975-76 and 1976-77 at Roanoke Rapids, NC.

more, *C. crotalariae* apparently does not colonize and produce MS in plant residues incorporated in soil after harvest of peanut or crops commonly grown in rotation with peanut. *C. floridanum* was reported to be a good competitive saprophyte (7), but we believe that *C. crotalariae* is a poor competitive saprophyte in soil.

Because low soil moisture has an unfavorable effect on colonization and decay of peanut roots by *C. crotalariae* (9), we believe that the absence of adequate soil moisture during the 1975 growing season accounts for the absence of CBR in Florigiant peanut and the failure of MS populations to increase in soil planted to this host. Rainfall was below normal during most of the 1976 growing season, but the distribution was such that periods of extreme low soil moisture were short. We believe that the soil moisture in 1976 was adequate for colonization of susceptible host roots and production of MS of *C. crotalariae*.

Host crops are important determinants of the magnitude of increase of MS populations in soil, as shown by the influence of peanut cultivars and rotational crops during the 1976 growing season. Of the crops evaluated, Florigiant peanut resulted in the greatest increase in MS populations in soil. Florigiant sustains severe CBR damage with as few as 0.5 MS per gram of soil, whereas Argentine and NC3033 remain resistant up to inoculum densities of 50 MS per gram of soil (8). Although Argentine and NC3033 appear to have similar sensitivity to MS densities, our results indicate that MS densities increase significantly ($P = 0.05$) in soil planted to Argentine but not NC3033.

Of the crops commonly rotated with peanuts (ie, soybean, tobacco, cotton, and corn), only soybean became infected by *C. crotalariae* and allowed an increase in MS populations in soil. In similar experiments with microplots containing artificially-infested field soil, Sartorato (12) found increased densities of MS in soil at harvest of Ransom and Forrest soybeans. Although yields of both cultivars were significantly ($P = 0.05$) suppressed when grown in infested soil, *C. crotalariae* has not been reported to suppress growth and yield of soybean in naturally-infested fields. As indicated by Krigsvold and Griffin (6), soybean cropping appears to be an important means of maintaining or increasing populations of *C. crotalariae* MS in peanut fields.

C. crotalariae overwinters in soil as MS (5,10), and temperatures during winter months are a primary determinant of their longevity, according to our data and that of others (3,14). During relatively mild winters, MS populations in soil exhibit almost no significant change. During cold winters, however, when soil water in the plow layer freezes or when temperatures remain at or below 5 C for 4–5 wk, the numbers of viable MS in soil are markedly reduced.

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