Influence of Soil Temperature and Moisture on the Severity of Cylindrocladium Black Rot in Peanut

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.

Paper No. 5165 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N. C.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named, nor criticism of similar ones not mentioned.

The authors thank Anne Harvey and Lori Hume for technical assistance and D. K. Cassel for assistance in soil moisture and texture determinations.

Accepted for publication 10 March 1977.

ABSTRACT

PHIPPS, P. M., and M. K. BEUTE. 1977. Influence of soil temperature and moisture on the severity of Cylindrocladium black rot in peanut. Phytopathology 67:1104-1107.

A soil temperature of 25 C and moisture content near field capacity were most conducive for infection and rot of peanut roots by *Cylindrocladium crotalariae*. Less root infection and rot occurred in soil at 20 C and 30 C, and no measurable disease resulted in soil at 35 C. The response of plants in soil at 35 C for 9 hr during daylight, then 25 C for the remainder of each day was similar to that obtained at a constant soil temperature of 35 C. A lower degree of root infection and rot resulted at 20, 25, and 30 C when soil was irrigated only after the moisture content reached a level near the wilting point of plants. In experiments conducted with artificially-infested field soil, the survival of *C. crotalariae* microsclerotia in soil was not affected either by the irrigation regime or by temperature.

Additional key words: *Arachis hypogaea* 'Florigiant', *Calonectria crotalariae*.

Cylindrocladium black rot of peanut is caused by the soilborne fungus, *Cylindrocladium crotalariae* (Loos) Bell and Sobers (3). Microsclerotia (ms) of *C. crotalariae* are thought to be the primary propagule that enables long-term survival in naturally-infested soil (6, 11). The annual increase in incidence of this disease in peanut fields in North Carolina and Virginia has necessitated an intensive effort to develop strategies for disease control. The absence of effective agricultural chemicals for disease control (2, 10) has increased efforts to identify resistant cultivars of peanut (5, 12).

Effective screening of plants in a breeding program and subsequent selection of resistant genotypes requires knowledge of conditions conducive for disease development. The current investigation was made to determine the optimum soil temperature and moisture for development of Cylindrocladium black rot in peanut under greenhouse conditions.

MATERIALS AND METHODS

Inoculum preparation and infestation of soil.—Microsclerotia of five pathogenic isolates of *C. crotalariae* were produced in a liquid medium as described by Rowe et al. (11). After 8-10 wk of incubation, ms larger than 74 μm were separated from fungal mycelium and suspended in water as previously reported (6). The density of ms in suspension was then determined by counting the number of ms in six, 1-ml samples.

Soil (fine sandy loam) was collected from the A horizon of a peanut field where Cylindrocladium black rot had never been observed and *C. crotalariae* was not detected in soil assays by the elutriation method (6). Quantities of soil weighing 1,700 g (1,618 g on a dry wt basis) were infested with 16,180 ms from the standardized suspension to provide an inoculum density of 10 ms/g soil. Each portion of soil was mixed in polyethylene bags for 5 min before being dispensed into 15-cm-diameter plastic pots with sealed bottoms.

Control of soil temperature and moisture.—Pots were placed in separate water-bath tanks equipped to maintain soil temperatures of 20, 25, 30, and 35 C (± 1.5 C). Ambient air temperatures, which ranged from 25 C to 40 C, were recorded daily with a maximum-minimum thermometer.

Soil moisture content at field capacity (FC) was 9% (w/w) as determined by the method described by Daubenmire (4). Based on results in a preliminary study, a soil moisture content of 3% was determined to be near the wilting point (WP) of peanuts. Moisture determinations of soil at pressures of 0.1, 0.33, and 15.0 bars were 11.97, 5.10, and 2.49% moisture content, respectively, with the pressure membrane apparatus method (7).

Two soil moisture regimes were maintained at each soil temperature. The first involved irrigating soil to FC (9% w/w) once daily during the first 4 wk of the experiment, then twice daily during the remainder of the experiment. In the second regime, soil was irrigated to field capacity

TABLE 1. Response of the peanut cultivar Florigiant to *Cylindrocladium crotalariae* in artificially-infested soil maintained at various temperatures and under two soil moisture regimes[w]

Soil treatment	Moisture regime[x]	Root rot severity index[y]	Percent recovery of *C. crotalariae*[z]
20 C:			
Noninfested	Dry	0.3 a	0
	Wet	0.8 a b	0
Infested	Dry	1.2 b	40
	Wet	2.0 c	56
25 C:			
Noninfested	Dry	0.3 a	0
	Wet	1.0 b	0
Infested	Dry	1.4 b	82
	Wet	3.2 c	100
30 C:			
Noninfested	Dry	0.3 a	0
	Wet	0.6 a b	0
Infested	Dry	0.3 a	18
	Wet	1.0 b	33
35 C:			
Noninfested	Dry	0.3 a	0
	Wet	0.5 a	0
Infested	Dry	0.3 a	0
	Wet	0.6 a	2
35 C day/25 C night:			
Noninfested	Dry	0.3 a	0
	Wet	0.6 a	0
Infested	Dry	0.3 a	2
	Wet	0.6 a	0

[w]Means (average of five replicates of three plants each) in columns followed by the same letter(s) are not significantly different at $P = 0.05$ according to Duncan's new multiple range test.

[x]Dry: Soil moisture was brought to field capacity after the moisture content reached a level near the wilting point of plants. Wet: Soil moisture was brought to field capacity daily.

[y]Severity of root rot rated on a scale from 0 (no visible damage) to 5 (completely destroyed) 9 wk after planting.

[z]Percent of tap root tissue biopsies (three/plant) yielding *C. crotalariae* in culture.

TABLE 2. Populations of *Cylindrocladium crotalariae* microsclerotia in artificially-infested soil planted to peanuts and maintained at various temperatures and under two soil moisture regimes[a]

Soil temperature	Soil moisture regime[b]	Microsclerotia/g soil[c]		
		Soil fraction	Debris fraction	Total
20	Dry	10.3	0.6	10.9
	Wet	9.7	1.2	10.9
25	Dry	9.3	0.7	10.0
	Wet	12.7	1.8	14.5
30	Dry	8.8	1.3	10.1
	Wet	10.2	1.9	12.1
35	Dry	7.2	0	7.2
	Wet	10.4	0	10.4
35-25[d]	Dry	7.9	0.3	8.2
	Wet	7.5	0	7.5

[a]Data are the average of two replicate samples of soil assayed by the elutriation procedure 9 wk after infesting soil and planting peanuts.

[b]Dry: soil moisture was brought to field capacity after the moisture content reached a level near the wilting point of plants. Wet: soil moisture was brought to field capacity daily.

[c]Soil fraction: particles collected on 38-μm sieve during elutriation of soil. Debris fraction: plant debris collected on 425-μm sieve and blended for 2 min in a Waring Blendor.

[d]Soil temperature was 35 C for 9 hr during daylight, then at 25 C for the remainder of each day.

after reaching a moisture content near the WP (3% w/w) of plants. Hereafter, the two moisture regimes are referred to as wet and dry soil, respectively. In each regime, deionized water was used to prevent the accumulation of soluble salts in soil. Since the plastic pots were of uniform weight (130 ± 5 g), soil moisture content was measured gravimetrically with marginal error at least twice daily during each experiment. At intervals of 2, 4, and 6 wk after planting peanuts, plants from one replicate of each moisture regime at the various temperatures were washed free of soil and weighed to permit correction of the weights used to maintain each moisture regime.

Experimental design and evaluation of disease severity.—Sixteen replicates of pots containing infested and noninfested soil were placed into each of five soil temperature tanks. Three, 3-day-old seedlings of the peanut cultivar, Florigiant, were planted in each pot. Soil was watered to FC and covered with a circular sheet of polyethylene to reduce loss of water by evaporation. Small slits were cut in the polyethylene to permit emergence of plants. Uniform emergence of plants was obtained by maintaining soil temperature at 28 C and moisture near FC during the 1st wk after planting. Following this period, single temperature tanks were set to maintain soil temperatures of 20, 25, 30, and 35 C. Two additional tanks were set at 25 and 35 C, respectively, to permit placement of pots at 35 C for 9 hr during daylight, then at 25 C for the remainder of each day. Plants were grown under natural light in the greenhouse.

Plant growth (fresh weight), the severity of root rot, and the occurrence of perithecia of *Calonectria crotalariae* were recorded 8 wk after planting. The severity of root rot was evaluated on a scale of 0 to 5 (0 = no visible damage, 5 = completely decayed) as illustrated by Rowe and Beute (9). Root infection by *C. crotalariae* was determined by collecting nine biopsy tissue samples from roots of plants in each replicate (three per plant), surface-sterilizing tissues by treatment for 1 min in 0.5% NaClO, then placing the tissues on a culture medium developed for isolation of *C. crotalariae* (6). The number of ms surviving in soil at the end of each experiment was determined by elutriation (6) of two replicate subsamples of infested soil from each treatment.

RESULTS

Root rot was most severe when plants were grown in wet soil at 25 C (Table 1). Comparison of plant growth in wet and dry soil at 25 C and the frequency of recovery of the pathogen by root tissue biopsy confirmed the higher pathogenic propensity of *C. crotalariae* in the infested wet soil. Perithecia of *Calonectria crotalariae* were abundant on necrotic tissues at the crowns of all plants in the infested wet soil, whereas none was found on plants in the infested dry soil.

Soil temperatures of 20 C and 30 C resulted in moderate and low root rot severities, respectively, in the infested wet soil (Table 1). At each temperature, a lower degree of root rot resulted in the infested dry soil. Only a few perithecia developed on plants in the infested wet soil at 20 C, whereas none was found on plants in the dry soil at 20 C nor in either wet or dry soil at 30 C. Plant growth was not significantly ($P = 0.05$) reduced in infested soils at either 20 or 30 C. Biopsied tissues from plants indicated more root infection by *C. crotalariae* occurred in the infested wet soil at both temperatures. Comparison of data on infection and root rot at 20 C and 30 C indicated that 20 C was more favorable than 30 C for disease development.

At 35 C, plant growth in infested soil was not significantly different ($P = 0.05$) from that in noninfested soil regardless of the soil moisture regime. The absence of root rot and the low frequency of recovery of *C. crotalariae* from roots indicated that 35 C is unfavorable for infection. Similar results were obtained in soils having a day temperature of 35 C and night temperature of 25 C. Neither the soil temperature nor the soil moisture regime had a marked effect on survival of ms (Table 2).

In another experiment of similar design, populations of ms in a naturally-infested loamy sand prior to planting ranged from 1.1 to 2.0 ms/g soil based on assays of four replicate subsamples by the elutriation method. Check treatments at each temperature consisted of the same soil steamed for 30 min at 82 C. No viable ms of *C. crotalariae* were detected in soil after steaming. Disease severity in the naturally-infested soil was strikingly similar to that obtained in the previous experiment with artificially infested soil. Root rot was most severe in wet soil at 25 C. Moderate to low root damage occurred in wet soil at 20 C and 30 C. At each temperature, root rot was less severe in dry soil. No root rot nor growth reduction were detectable in plants grown in soil at 35 C or in soil having 35/25 C, day/night temperatures.

DISCUSSION

Soil temperature and soil moisture both play an important role in determining the severity of Cylindrocladium black rot in peanut. *Cylindrocladium crotalariae* was most aggressive on peanut in soil at a temperature of 25 C and moisture level near FC. Soil temperatures of 20 C and 30 C were less favorable for root infection and rot. At all three temperatures, the pathogen was less aggressive in soil allowed to dry to near the WP of plants before irrigation to FC. The observed absence of diseased roots and the low level of root infection at a soil temperature of 35 C were contrary to the effect of soil temperature on severity of Cylindrocladium black rot in peanut reported by Bell (1). He found damage caused by *C. crotalariae* increased with increasing soil temperatures from 15 to 25 C and thereafter damage did not change significantly up to 40 C. The inherent differences in isolates of *C. crotalariae* from Georgia, the use of fumigated soil infested with comminuted oat grains colonized by the fungus, the wounding of roots during inoculation, and/or absence of uniform soil moisture of each temperature may account for the wide temperature range reported favorable for development of this disease. We believe that our results enable a more precise evaluation of temperature effects since artificially-infested and naturally-infested field soil were used, and soil moisture was carefully managed at each temperature.

The optimum temperature for vegetative growth of *C. crotalariae* on potato-dextrose agar is 26-28 C (3). At 20 C the growth rate is approximately 50 percent of that observed at 25 C, and essentially no growth by North Carolina isolates occurs at temperatures of 35 C and above (8). The results of the current study on the effect of

soil temperature on disease development correlate well with the reported effect of temperature on vegetative growth of *C. crotalariae* in axenic culture. Soil temperatures over the range of 20 to 35 C had no marked effect on inoculum survival; reduced ms germination and/or mycelial growth may thus account for the low levels of disease at 20 and 30 C and no disease at 35 C.

Field observations of this disease in North Carolina since 1971 indicate that heavy rainfall in June and/or July followed by extended dry periods in August and September contribute to severe disease losses. Similar observations on the severity of Cylindrocladium black rot in peanuts have been reported in Georgia by Bell (1), who proposed that this disease is most severe when a period of excessively high soil moisture is followed by a period of extreme moisture stress and high temperature. In North Carolina, a period of high rainfall early in the growing season is believed necessary for severe root rot since most peanut field soils are well-drained and sandy in texture. The subsequent period of moisture stress is thought to enhance the expression of above ground symptoms due to the absence or limited number of functional roots after infection and root rot.

LITERATURE CITED

1. BELL, D. K. 1967. Effects of soil temperature and plant age on the severity of Cylindrocladium rot of peanut. Plant Dis. Rep. 51:986-988.
2. BELL, D. K., B. J. LOCKE, and S. S. THOMPSON. 1973. The status of Cylindrocladium black rot of peanut in Georgia since its discovery in 1965. Plant Dis. Rep. 57:90-94.
3. BELL, D. K. and E. K. SOBERS. 1966. A peg, pod, and root necrosis of peanuts caused by a species of Calonectria. Phytopathology 56:1361-1364.
4. DAUBENMIRE, R. F. 1947. Plants and environment. John Wiley and Sons, New York. 424 p.
5. PHIPPS, P. M., and M. K. BEUTE. 1977. Sensitivity of susceptible and resistant peanut cultivars to inoculum densities of Cylindrocladium crotalariae microsclerotia in soil. Plant Dis. Rep. 61:300-303.
6. PHIPPS, P. M., M. K. BEUTE, and K. R. BARKER. 1976. An elutriation method for quantitative isolation of Cylindrocladium crotalariae microsclerotia from peanut field soil. Phytopathology 66:1255-1259.
7. RICHARDS, L. A. 1947. Pressure membrane apparatus construction and use. Agric. Eng. 28:451-454.
8. ROWE, R. C., and M. K. BEUTE. 1975. Ascospore formation and discharge by Calonectria crotalariae. Phytopathology 65:393-398.
9. ROWE, R. C., and M. K. BEUTE. 1975. Variability in virulence of Cylindrocladium crotalariae isolates on peanut. Phytopathology 65:422-425.
10. ROWE, R. C., M. K. BEUTE, J. C. WELLS, and J. C. WYNNE. 1974. Incidence and control of Cylindrocladium black rot of peanuts in North Carolina during 1973. Plant Dis. Rep. 58:348-352.
11. ROWE, R. C., S. A. JOHNSTON, and M. K. BEUTE. 1974. Formation and dispersal of Cylindrocladium crotalariae microsclerotia in infected peanut roots. Phytopathology 64:1294-1297.
12. WYNNE, J. C., R. C. ROWE, and M. K. BEUTE. 1975. Resistance of peanut genotypes to Cylindrocladium crotalariae. Peanut Sci. 2:54-56.