Soil Components That Affect Severity of Cylindrocladium Black Rot on Peanuts

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ABSTRACT

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Increases in densities of actinomycetes and bacteria and increases in copper levels in soil from peanut, soybean, and corn fields were associated with decreases in root rot severity on peanut seedlings after infestation with microsclerotia of *Cylindrocladium crotalariae*. Root rot was more severe after infestation of untreated soil from corn fields than from soybean fields. Peanuts grew better after adding actinomycetes cultured from soils at one location than with actinomycetes from soils at a second location, but root rot severity was not affected by actinomycetes from two locations. Actinomycete density in *C. crotalariae*-infested soil in microplots was greater with soybeans than with peanuts or corn. Severity of Cylindrocladium root rot was not affected when actinomycetes from microplots were used to amend soil, but the actinomycete populations from peanut and soybean microplot soils enhanced plant growth compared with those from corn microplot soils.

Additional key words: *Arachis hypogaea*, suppressive soil

Soil microflora can have dramatic effects on the pathogenicity of *Cylindrocladium crotalariae* (Loos) Bell & Sobers. Tobacco (*Nicotiana tabacum* L.) transplanted into fumigated soil and inoculated with *C. crotalariae* developed root rot (11). However, tobacco grown in untreated peanut (*Arachis hypogaea* L.) field soils in the greenhouse (B. A. Hadley and M. K. Beute, *unpublished*) and in untreated field microplots (8) was not susceptible to *C. crotalariae*.

Barron and Phipps (3) found that different levels of peanut root rot occurred in two soils infested at the same inoculum density of *C. crotalariae*. We showed that microsclerotia of *C. crotalariae* in soil following soybeans (*Glycine max* (L.) Merr.) were less efficient at inducing peanut root rot than microsclerotia in soil following peanuts (5). Symptoms of Cylindrocladium black rot no longer appeared on shoots, root rot severity was minimal, and inoculum density of *C. crotalariae* did not increase after two cycles (3–4 mo each) of greenhouse monoculture of peanuts or soybeans in soil from two *C. crotalariae*-infested fields in North Carolina (M. C. Black, *unpublished*). Decline of disease following monoculture under field conditions has been recognized for other host-pathogen systems (1).

We sought in this study to determine if severity of Cylindrocladium root rot on peanuts was affected by biotic soil components, and if so, whether fungi, bacteria, and/or actinomycetes were involved.

MATERIALS AND METHODS

Population densities. Populations of fungi, bacteria, and actinomycetes in various soils were determined by counting colonies on dilution plates. Cooled potato-dextrose agar amended with Tergitol surfactant (Union Carbide, Atlanta, GA) (1 ml/L) and streptomycin sulfate (0.1 g/L) before adding soil diluted in sterile water was used for fungi (13). For bacteria, soil diluted in saline (8.5 g of NaCl/L) was added to soil-extract agar amended with PCNB (0.05 g/L) (6). For detection of actinomycetes, soil was added to a phenol solution (7.8 ml/L), diluted in sterile water, and added to autoclaved glycerol-arginine-salt medium (adjusted to pH 6.5 with dilute HCl) amended with filter-sterilized cycloheximide (200 mg/L) (7). Plates were incubated at 25 C (±1.5).

Microplots. Microplots (76 cm in diameter) were established in April 1979 in a Norfolk loamy sand at the North Carolina State University (NCSU) Central Crops Research Station at Clayton (2). A composite of 12 isolates of *C. crotalariae* was used to infest soil in June 1979. Microplots had histories of rotation (Table 1) with susceptible Florigiant peanuts (F), resistant NC 3033 peanuts (N), soybeans (S) (a host), and corn (*Zea mays* L.) (C) (not a host) and were managed as described elsewhere (5). A four-character code is used in this paper to indicate the crop sequence used for the 1979–1982 growing seasons, with a dash (-) indicating that soil was sampled before planting in 1982 for fungi and bacteria. The crop sequences FFF-, NNN-, SNN-, and SSN- were compared for numbers of fungi and bacteria, and the sequences FFFF, NNNN, SSNS, and CCNC were compared for actinomycetes.

Soil sampling. About 500 g (±75) of soil was sampled using a soil probe (cores 15 × 2 cm) from each microplot. Samples were sieved (2.38-mm opening), mixed 1 min by shaking, and stored at 25 C (±1.5) in closed polyethylene bags until assayed. Soils sampled on 1 April 1982 were assayed for fungi and bacteria. Actinomycete population densities were estimated from soil sampled on 29 July 1982

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Table 1. Selected previous treatments in microplots where soil was sampled and assayed in this study for numbers of bacteria, actinomycetes, and fungi; for root rot severity in October 1981 on peanuts; and for inoculum density of *Cylindrocladium crotalariae* in January 1981 and 1982

Growing season				Inoculum density[a] (Jan. 1981)	Root rot severity[b] (Oct. 1981)	Inoculum density[a] (Jan. 1982)
1979	1980	1981	1982			
S[c]	S	N	S	17.2	0.1	3
S	N	N	···	12.3	0.0	7
F	F	F	F	19.1	4.8	40
N	N	N	N	15.6	2.1	15
C	C	N	C	7.4	0.3	3
Fisher's $LSD_{0.05}$				11.2	2.0	27
C.V.				65%	67%	100%

[a] Microsclerotia per cubic centimeter of soil.
[b] 0 = No lesions and 5 = completely rotted roots.
[c] F = susceptible Florigiant peanuts, N = resistant NC 3033 peanuts, S = soybeans (a host), and C = corn (not a host).

and 14 October 1982.

Fields not infested with *C. crotalariae* were sampled at Byrd Farm, Clarkton, NC, on 3 November 1982 and at NCSU Central Crops Research Station, Clayton, NC, on 8 November 1982. A total of 24 samples were collected. At each of the two locations, four fields, each following peanuts, corn, or soybeans, were sampled. Each sample of 3 kg (±0.25) was uniformly collected from an area 15 × 15 m with a soil probe (cores 15 × 2 cm). Three loamy sand, six sandy loam, and three fine sandy loam soils were sampled at Clarkton after harvesting corn (disked twice) and peanuts and before harvesting soybeans. At Clayton, nine loamy sand, one sandy clay loam, and two sandy loam soils were sampled after harvesting corn (before disking) and peanuts and before harvesting soybeans. Samples were processed as described before. Dilution plates for uninfested soils were poured for fungi on 15 November 1982, for bacteria on 17 November and 2 December 1982, and for actinomycetes on 11 November and 7 December 1982.

Infestation with microsclerotia. Each uninfested field soil sample was subdivided into four portions. The first portion was analyzed for soil fertility and the second examined for infestations of plant-parasitic nematodes. The third portion was fumigated with 98% methyl bromide and 2% chloropicrin at a rate of 49 g/m^2 for 48 hr. The fourth soil portion was left untreated. Fumigated and untreated soils were aired for 4 days with daily mixing. Soil was infested with *C. crotalariae* (35 microsclerotia per cubic centimeter) (9) and granular *Rhizobium* (5 g/L of soil) (cowpea cross-inoculation group) (Nitragin Co., Goldsboro, NC). Three 10-cm-diameter plastic pots were filled with each soil treatment. Each pot was then placed inside a larger 15-cm-diameter clay pot. This served as a splash barrier during waterings to avoid cross-contamination. Three-day-old seedlings of NC 3033 peanuts were transplanted into the pots and thinned to two plants per pot 4 days later. Plant height, root rot severity (0 = no lesions and 5 = completely rotted roots) (4), and mass of oven-dry shoots and roots were recorded at 6 wk.

Response to actinomycetes from microplot soil. After culturing for 2 wk, all actinomycete colonies (1–4 mm in diameter) were cut out with a minimum of agar and blended in sterile deionized water for 1 min at high speed. Volume of the suspension was adjusted to the equivalent of seven colonies per 50 ml. Fifty milliliters of the suspension was pipetted into 1,400 cm^3 of steamed sand:steamed sandy loam (1:1). After 1 wk, *Rhizobium* inoculum and *C. crotalariae* at 35 microsclerotia per cubic centimeter were mixed in soil for 1 min. Soil was placed in 15-cm-diameter clay pots placed inside 22-cm-diameter clay pots in the greenhouse at 25 C (±2). Three-day-old seedlings of NC 3033 peanuts were transplanted and thinned to three plants per pot 4 days later. Plant height, mass of oven-dry shoots and roots, and root rot severity were recorded at 7 wk. Actinomycetes from crop sequences of FFFF, NNNN, SSNS, and CCNC were compared.

Response to actinomycetes from field soil. Twenty-eight actinomycete colonies were selected randomly from dilution plates for each soil sample, representing populations from peanut, soybean, and corn fields. Colonies were blended for 1 min at high speed in 80 ml of sterile deionized water. An equivalent of 8.75 colonies per 25 ml of the suspension was added to 1,400 cm^3 of untreated loamy sand from Clayton (not infested with *C. crotalariae*) and assayed as described.

RESULTS

Population densities of fungi and bacteria in microplots did not differ significantly in April 1982 among rotation treatments (Fig. 1). In August 1982, however, there were more actinomycetes in microplots planted with soybeans in 1982 (SSNS) than in plots planted with peanuts (FFFF, NNNN) or corn (CCNC) (Fig. 1). Actinomycete population densities in soil in October 1982 were similar for Florigiant and corn, greater for NC 3033 than for Florigiant, and greater for soybean than for

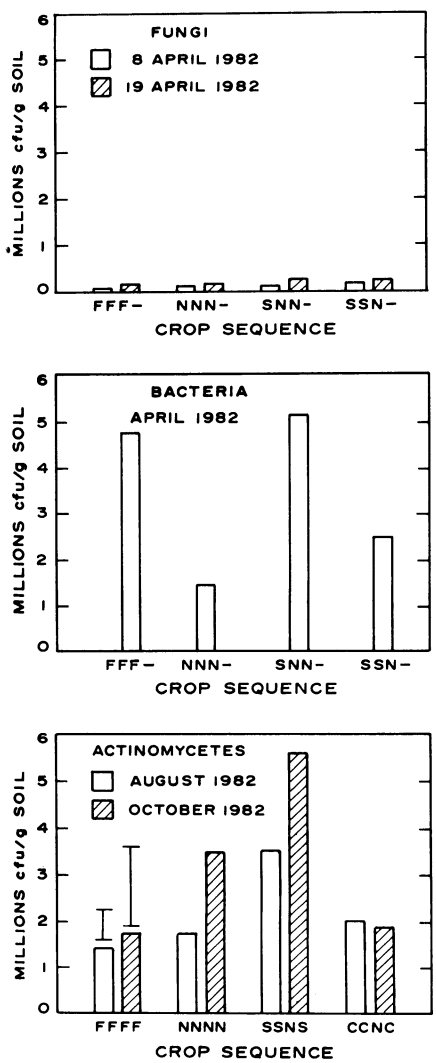

Fig. 1. Densities in 1982 of fungi, bacteria, and actinomycetes in microplot soil infested in 1979 with *Cylindrocladium crotalariae*. Susceptible Florigiant peanuts (F), resistant NC 3033 peanuts (N), soybeans (S), or corn (C) were grown in 1979–1982 in sequences indicated by a code, with a dash indicating soil was sampled for fungi and bacteria before planting in 1982. Bar represents Fisher's least significant difference (LSD) at $P = 0.05$.

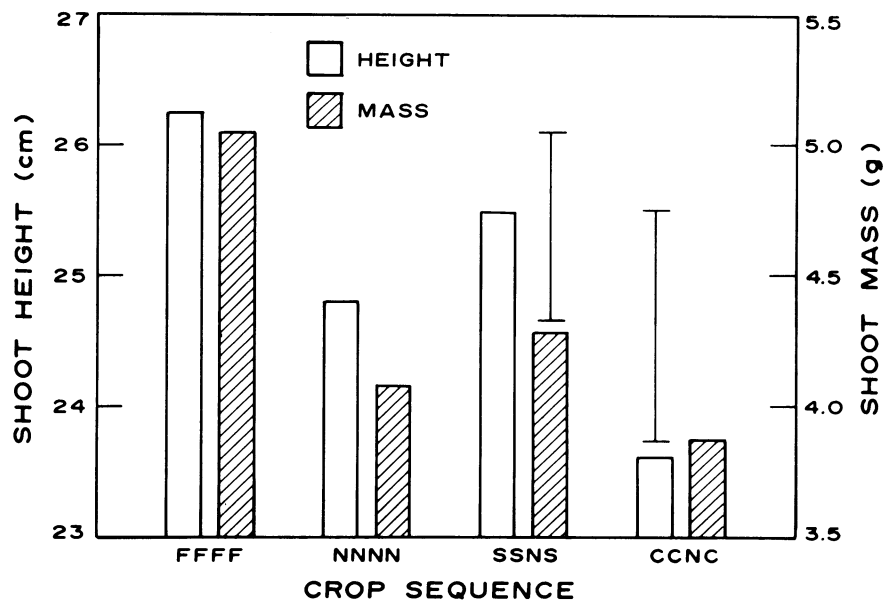

Fig. 2. Height and mass of peanut shoots grown in soil uniformly infested with *Cylindrocladium crotalariae*. Treatments were different actinomycete populations isolated at harvest in October 1982 from microplot soil following different crop sequences. Susceptible Florigiant peanuts (F), resistant NC 3033 peanuts (N), soybeans (S), or corn (C) were grown in 1979–1982 as indicated by a four-character code. Bar represents Fisher's least significant difference (LSD) at $P = 0.05$.

Florigiant, NC 3033, or corn (Fig. 1).

The height and mass of peanut shoots were both affected by actinomycete populations originating from different crop sequences in microplots (Fig. 2). The severity of peanut root rot was not affected. The height of shoots grown in soil amended with actinomycetes from microplot soil following growth of Florigiant peanuts or soybeans in 1982 was greater than that following corn. The mass of shoots was greater when grown in soil amended with actinomycetes from microplot soil following growth of Florigiant peanuts in 1982 than following soybeans, NC 3033 peanuts, or corn.

No significant differences were found among peanut, soybean, and corn field soils after one cropping sequence for densities of fungi, bacteria, actinomycetes, or nematodes. Numbers of microflora organisms per unit of soil were of the same order of magnitude when compared with microplots infested with *C. crotalariae*.

The severity of peanut root rot was not affected by amendments with actinomycetes isolated from two locations in North Carolina and following three crops (Table 2). The mass of peanut shoots was greater in untreated soil amended with actinomycete populations from Clarkton than in similar soil amended with actinomycetes from fields near Clayton. The differences were not statistically significant, but the mass of roots tended to be greater with actinomycetes cultured from Clarkton than from Clayton soils ($P = 0.14$). The mass of roots tended to be greater with actinomycetes in soil following peanuts than following corn ($P = 0.09$). The height of shoots also tended to be greater with actinomycetes from Clarkton than from Clayton soils ($P = 0.11$).

Root rot was more severe and dry mass of shoots was less in *C. crotalariae*-infested, fumigated soil than in infested, untreated soil at both locations (Fig. 3). The experiment was not designed to compare locations, but mean root rot severity in untreated soils from Clarkton was consistently greater than mean root rot in untreated soils from Clayton. Root rot severity in untreated soil from Clarkton was greater if soil was from corn fields than from soybean fields. Root rot severity rank in untreated soils was the same for Clayton and Clarkton, but differences among the untreated soil treatments from Clayton were not significant (Fig. 3).

There was a significant crop × fumigation interaction among field soils from Clayton. The ranking of root rot severity among untreated peanut, soybean, or corn field soils was the opposite of that in fumigated soils (Fig. 3).

Biotic and abiotic soil factors were related to peanut root rot severity as revealed by multiple linear regressions using Mallow's C_p statistic (12) (Table 3). Root rot was less severe at higher bacterium and actinomycete densities when considering only biotic factors in soils from Clarkton. Copper index value replaced bacteria in the model and increased the coefficient of determination (R^2) when abiotic soil factors were also considered. Sixty-four percent of the variation in root rot severity in Clarkton

Table 2. Response of peanut seedlings grown in a greenhouse in soil concomitantly infested with *Cylindrocladium crotalariae* and amended with actinomycetes isolated from soil from two locations following peanuts, soybeans, or corn

Variable	Main effect of soil location[a]	Mean response	Main effect of previous crop	Mean response
Shoot mass[b]	Clarkton	5.77	Peanut	5.60
	Clayton	5.29	Soybean	5.45
	$P>F$	0.01	Corn	5.37
	$LSD_{0.05}$	0.48	$P>F$	0.75
Root mass[b]	Clarkton	1.40	Peanut	1.42
	Clayton	1.32	Soybean	1.37
	$P>F$	0.14	Corn	1.27
			$P>F$	0.09
Shoot height[c]	Clarkton	19.00	Peanut	18.80
	Clayton	18.40	Soybean	19.10
	$P>F$	0.11	Corn	18.10
			$P>F$	0.22
Root rot severity[d]	Clarkton	1.00	Peanut	0.90
	Clayton	1.00	Soybean	1.10
	$P>F$	0.55	Corn	1.00
			$P>F$	0.59

[a] Clarkton, NC: three loamy sand, six sandy loam, and three fine sandy loams soils. Clayton, NC: nine loamy sand, one sandy clay loam, and two sandy loam soils.
[b] In grams (oven-dry).
[c] Distance (cm) from cotyledons to growing point of dominant shoot.
[d] Caused by *C. crotalariae*; 0 = no lesions and 5 = completely rotted roots.

Table 3. Variation in peanut root rot severity caused by *Cylindrocladium crotalariae* that was related to variation in biotic and abiotic soil components

Type of variables[a]	Equation[b]	R^2	N[c]	$P>F$
Soil from Clarkton[d]				
Biotic	$Y = 3.76 - 11.4 \times 10^{-7}$ (AD) $- 0.9 \times 10^{-7}$ (BD)	0.54	21	<0.01
Biotic and abiotic	$Y = 3.69 - 8.0 \times 10^{-7}$ (AD) $- 3 \times 10^{-2}$ (Cu)	0.64	21	<0.01
Soil from Clayton[d]				
Biotic and abiotic	$Y = 1.65 - 2.6 \times 10^{-7}$ (AN)	0.16	36	<0.02

[a] Biotic = number of fungi, bacteria, and actinomycetes per gram of soil. Abiotic = soil analyses by N.C. Department of Agriculture, Raleigh.
[b] Multiple linear regression analyses using Mallow's C_p statistic.
[c] N = number of observations.
[d] Clarkton, NC: three loamy sand, six sandy loam, and three fine sandy loam soils. Clayton, NC: nine loamy sands, one sandy clay loam, and two sandy loam soils. AN and AD = actinomycetes per gram of soil in November or December 1982, respectively; BD = bacteria per gram of soil in December 1982; and Cu = copper index.

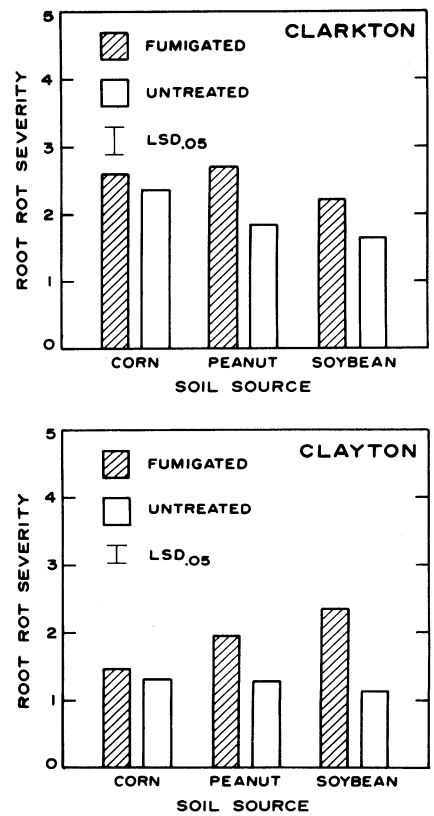

Fig. 3. Root rot severity on NC 3033 peanuts in soils from peanut, soybean, or corn fields (near Clarkton and Clayton, NC). Soils were fumigated or left untreated before being infested with *Cylindrocladium crotalariae* at 35 microsclerotia per cubic centimeter. Bar represents Fisher's least significant difference (LSD) at $P = 0.05$.

soils could be explained by actinomycete density and the copper index value. Increases in density of actinomycetes were related to decreases in root rot severity in Clayton soils, but the R^2 values were low (Table 3).

DISCUSSION

We showed previously that microsclerotia of *C. crotalariae* were less efficient at inducing Cylindrocladium root rot on peanuts following soybeans than on peanuts following peanuts or corn (5). In this study, there were more actinomycetes in soybean-microplot soil infested with *C. crotalariae* than in peanut- or corn-microplot soils (Fig. 1).

Increases in densities of both actinomycetes and bacteria in field soils were related to decreases in peanut root rot severity after artificial infestation with *C. crotalariae* (Table 3).

There were fewer fungi in soil than either bacteria or actinomycetes (Fig. 1), which agrees with previous work (1). The treatments in this study did not affect fungus populations (Fig. 1).

Soil microflora are thought to contribute to plant growth as sources of growth hormones and organic and inorganic nutrients by affecting availability and conversion of materials to a useful form and through suppression of major and minor pathogens (1,14,16). Actinomycetes isolated from soils following different crops had significant effects on growth of peanuts (Fig. 2, Table 2). However, Cylindrocladium root rot severity on peanuts was not affected (Table 2). Actinomycetes isolated from soil following susceptible Florigiant peanuts were the most favorable and populations after corn were the least favorable for growth of peanuts (Fig. 2).

Plant growth was greater in greenhouse soil amended with actinomycetes isolated from soils near Clarkton than with actinomycetes from soils near Clayton (Table 2). Average peanut yields are greater in the Clarkton area than near Clayton and microflora differences may be involved. Rovira (10) estimated that as much as 20% of a plant's production potential can be lost because of unfavorable microbiological situations.

Biotic and abiotic soil components were associated with different Cylindrocladium root rot severities on peanuts in this study (Fig. 3, Table 3). Crop rotation and field location were related to quantitative and/or qualitative differences in microorganism populations (Figs. 1 and 2, Tables 2 and 3). Other work has shown that source of soil influenced severity of Cylindrocladium root rot on peanuts (3).

Many soil chemistry variables are highly correlated (15) and more work is needed to determine whether copper (Table 3) or other correlated soil factors influence root rot severity. Soil particle size was not evaluated in this study, but higher clay content in soils was possibly related to the lower root rot severities in Clayton soils (Fig. 3).

Suppressive soils are likely to have high densities of microorganisms that include species favorable for peanut growth (1,10,14). Fungistasis of microsclerotia of *C. crotalariae* might delay or prevent germination because of competition from bacteria for exudates from seeds, roots, and pods (1). Bacteria are generally limited under dry and warm conditions, but actinomycetes would do well if concentrations of organic matter were high (1). High organic matter in soil would also improve the abiotic quality of soil, including longer retention of many nutrients (15).

We surmise, on the basis of this and another study (5), that soils are relatively conducive to Cylindrocladium root rot of peanuts when only corn and peanuts are used in rotation. We would expect a more suppressive soil where soybeans, corn, and peanuts are rotated, winter legume cover crops are grown, and both major and minor nutrients are adequate.

LITERATURE CITED

1. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN. 433 pp.
2. Barker, K. R., Daughtry, B. I., and Corbett, D. W. 1979. Equipment and techniques for establishing field microplots for the study of soilborne pathogens. J. Nematol. 11:106-108.
3. Barron, J. A., and Phipps, P. M. 1983. Interaction of dinitramine and dinoseb with *Cylindrocladium crotalariae* and the Cylindrocladium black rot (CBR) disease of peanut. Peanut Sci. 10:101-106.
4. Black, M. C., and Beute, M. K. 1984. Relationships among inoculum density, microsclerotium size, and inoculum efficiency of *Cylindrocladium crotalariae* causing root rot on peanuts. Phytopathology 74:1128-1132.
5. Black, M. C., and Beute, M. K. 1984. Effects of rotations with susceptible and resistant peanuts, soybeans, and corn on inoculum efficiency of *Cylindrocladium crotalariae* on peanuts. Plant Dis. 68:401-405.
6. Farley, J. D., and Lockwood, J. L. 1968. The suppression of actinomycetes by PCNB in culture media used for enumerating soil bacteria. Phytopathology 58:714-715.
7. Panthier, J. J., Diem, J. L., and Dommergues, Y. 1979. Rapid method to enumerate and isolate soil actinomycetes antagonistic towards rhizobia. Soil Biol. Biochem. 11:443-445.
8. Phipps, P. M., and Beute, M. K. 1979. Population dynamics of *Cylindrocladium crotalariae* microsclerotia in naturally-infested soil. Phytopathology 69:240-243.
9. Phipps, P. M., Beute, M. K., and Hadley, B. A. 1977. A microsclerotia-infested soil technique for evaluating pathogenicity of *Cylindrocladium crotalariae* isolates and black rot resistance in peanut. (Abstr.) Proc. Am. Phytopathol. Soc. 4:146.
10. Rovira, A. D. 1972. Studies on the interactions between plant roots and microorganisms. J. Aust. Inst. Agric. Sci. 38:91-94.
11. Rowe, R. C., and Beute, M. K. 1973. Susceptibility of peanut rotational crops (tobacco, cotton and corn) to *Cylindrocladium crotalariae*. Plant Dis. Rep. 57:1035-1039.
12. SAS Institute. 1982. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC. 584 pp.
13. Steiner, G. W., and Watson, R. D. 1965. Use of surfactants in the soil dilution and plate count method. Phytopathology 55:728-730.
14. Suslow, T. V., and Schroth, M. N. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72:111-115.
15. Tisdale, S. L., and Nelson, W. L. 1975. Soil fertility and fertilizers. 3rd ed. Macmillan, New York. 694 pp.
16. Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.