

# Survival of *Macrophomina phaseolina* Sclerotia in Soil: Effects of Soil Moisture, Carbon:Nitrogen Ratios, Carbon Sources, and Nitrogen Concentrations

O. D. Dhingra and J. B. Sinclair

Research Associate and Professor, respectively, Department of Plant Pathology, University of Illinois, Urbana 61801.

Supported in part by the Illinois Agricultural Experiment Station.

Accepted for publication 11 September 1974.

## ABSTRACT

Sclerotium populations of *Macrophomina phaseolina* declined rapidly under high soil moisture and in soils amended with glucose and NaNO<sub>3</sub> in different C:N ratios. At soil moisture of 60-100% moisture holding capacity, sclerotium populations declined 96-99% as compared with populations in dry soil (control). Sclerotium populations declined rapidly within 2-3 weeks in soils amended with glucose, sucrose, starch, or cellulose with the greatest reduction (81%) occurring with the addition of sucrose. In soils amended with sodium nitrate, *M. phaseolina* sclerotium populations decreased for 2-3 weeks after treatment, then

increased until at 7 weeks, populations exceeded the controls. Sclerotium populations declined more rapidly when soils were amended with glucose (1%, w/w air dry soil) and NaNO<sub>3</sub> mixtures giving C:N ratios of 10 and 20, than at 40 and 80. The sclerotium populations for all C:N ratios decreased by 58-61% compared with the control during the first week after treatment. Among all amendments the decrease in *M. phaseolina* populations was significantly correlated with increase in total microbial population in the soil.

Phytopathology 65:236-240

*Additional key words:* charcoal rot, cultural practices.

---

*Macrophomina phaseolina* (Tassi) Goid. [*M. phaseoli* (Maubl.) Ashby, *Rhizoctonia bataticola* (Taub.) Butler] causes seedling blight, charcoal rot and root rot of at least 400 plant species, usually under dry, warm conditions (28-35 C) (29). Mycelium of *M. phaseolina* grows to a limited extent in natural soils, but grows extensively in sand and partially-sterilized soils. Mycelial growth in soil increases with increased temp up to 35 C, but decreases with increasing soil moisture (19, 23). Although the mycelium is capable of limited growth in natural soils, it does not survive for more than 7 days (9, 10, 19, 21).

Sclerotia are the most important propagule for survival in soil. They are formed in host tissue and released into

the soil as host tissues decay (4, 18, 31). Soil populations of sclerotia increase if susceptible host crops are grown annually (21). *M. phaseolina* can survive as a saprophyte by colonizing dead organic substrates in the soil (12, 28, 35). However, the primary inoculum comes from either sclerotia free in the soil or embedded in host tissues (4, 22, 31).

This study reports the effects of soil moisture levels, amendments to give various carbon:nitrogen (C:N) ratios, various carbon (C) sources, and two NaNO<sub>3</sub> levels on the survival of *M. phaseolina* sclerotia in two soil types.

MATERIALS AND METHODS.—Two soil types

were used: a sandy-loam, consisting of 70% sand, 19% silt, and 11% clay, pH 7.4, total N of 0.036%, total organic matter of 1.2%, initial total microbial population of  $9 \times 10^7$ /g of soil, and a moisture-holding capacity (MHC) of 32%; and a silty-clay-loam, consisting of 9% sand, 61% silt, and 30% clay, pH 5.5, total N of 0.178%, total organic matter of 4.3%, initial microbial population of  $1.1 \times 10^8$ /g of soil, and a MHC of 60%. Soil analyses were made by the Department of Agronomy, University of Illinois, Urbana, and the Crop Chemical Testing Service, Arcola, Illinois. The soils were air-dried to 3% moisture and then screened through a 2-mm sieve.

Sclerotia were produced in soybean-seed-extract broth (100 g seeds/liter of water boiled for 15 minutes, strained through cheesecloth and supplemented with 2% sucrose). After 10 days at 30 C, cultures consisting of sclerotia and mycelium were homogenized in a VirTis mixer with sterile distilled water. The homogenate was filtered on a Whatman #42 filter paper and washed with sterile distilled water three times to remove excess broth. Fragments containing sclerotia were dried at 40 C for 24 hours, ground in a mortar and pestle, then screened through a 149- $\mu$  sieve to separate the individual sclerotia. Sclerotia were found to be viable when tested on chloroneb-mercury-rose bengal agar (CMRA), a selective medium for *M. phaseolina* (21), and Difco potato-dextrose agar supplemented with 30  $\mu$ g/ml streptomycin sulfate.

Dry sclerotia were added at a rate of 500 mg/kg of soil and mixed in a twin-arm mixer for at least 24 hours. Addition of sclerotia gave an approximate population of 680 sclerotia/g soil on an oven-dry weight basis, as determined by plating samples of the infested soil on CMRA plates in 20 replications.

Infested soils were amended with either different C sources or  $\text{NaNO}_3$  concentrations, or glucose plus  $\text{NaNO}_3$  in different C:N ratios. Carbon sources were glucose, sucrose, soluble starch and cellulose added at a rate equivalent in C to that provided by 1% glucose in air-dry soil (w/w).  $\text{NaNO}_3$  was added to give either 0.94 or 1.88 mg N/g (w/w) of air-dry soil. Amendments to provide C:N ratios of 10, 20, 40, and 80 were based on 1% glucose in air-dried soil (w/w) and  $\text{NaNO}_3$ . All amendments except cellulose were added as 20% solutions. Dry cellulose powder (Sigma Chemical Co., St. Louis) was mixed with soil samples in a twin-arm mixer for 4-5 hours. Nonamended soils served as controls. All soil samples were then adjusted to 33% MHC of the respective soils on an oven-dry weight basis.

Results from our studies showed that soil moisture influenced sclerotium survival. Samples of the two soil types were adjusted to 20, 40, 60, 80, and 100% MHC of the respective soil types without amendments.

All treatments were prepared in triplicate and placed in glass containers, covered with Parafilm to reduce moisture loss, and punctured to allow for air exchange, and stored at 30 C. Samples were taken at weekly intervals for 4 weeks, and a final sample at 7 weeks for population determination. Soil moisture was maintained at the original MHC levels (26). All experiments were performed twice.

Soil samples were air-dried for not more than 16 hours, crushed in a mortar and pestle, and 50 mg was sprinkled in five CMRA plates. If little or no colony growth was

recorded, a 100- to 400-mg soil sample was sprinkled in 10-40 CMRA plates. The soil plate method (34) using peptone-rose bengal agar (32) was used to determine fungal populations in soil samples. Bacterial and actinomycete populations were determined by a dilution plate method using 20 soil-extract-nutrient agar and 20 glucose-nitrate agar plates, respectively (32). The populations obtained were summed to obtain the total microbial population for each soil sample. Data were analyzed using a split-plot design and Fisher's LSD test (3). Calculated F values were significant.

**RESULTS.**—Results were similar for both soil types. Reductions in sclerotium populations of *M. phaseolina* due to high soil moisture appeared to be as much as with different soil amendments, if not more. Sclerotium populations decreased rapidly with increased soil moisture, and duration of incubation (Fig. 1). There was a 58-61 and 65-69% reduction in sclerotium populations below the control in sandy-loam and silty-clay-loam soils, respectively, at 60 to 100% MHC, at the 1-week sampling. At the 4- and 7-week sampling, there was a 90-96% and a 96-99% reduction, respectively, in both soil types. At the higher soil moistures, there was an accompanying 50 to 60% decrease in populations of other fungi, but a 5- to 6-fold increase in bacterial populations over the 7-week incubation period.

The largest decline of sclerotium populations in soils treated with various C sources occurred between 2-3 weeks (Fig. 2-A). Sucrose-amended soils had 81 and 85% fewer sclerotia than controls in sandy-loam and silty-clay-loam soils, respectively, at 7 weeks. After 4 weeks, sclerotium populations in starch- and glucose-amended soils increased, but remained below controls at 7 weeks.

Among soils amended with  $\text{NaNO}_3$ , maximum reduction occurred in 4 and 2 weeks at N concentrations

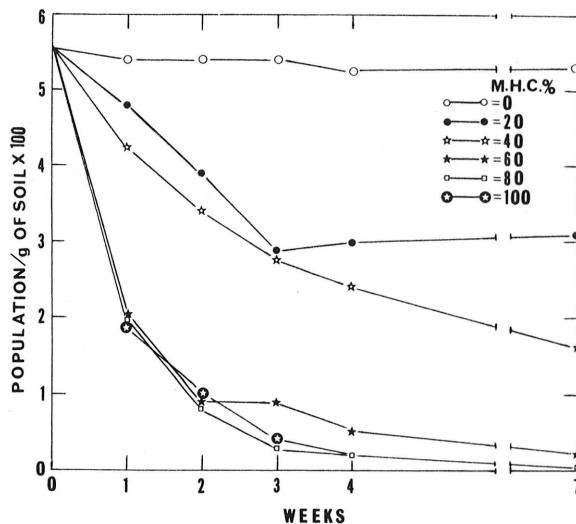


Fig. 1. Changes in the population of *Macrophomina phaseolina* over 7 weeks in sandy-loam soil at various percentages of moisture holding capacity (MHC%). Data based on two runs with three replications for each treatment. Fisher's LSD ( $P = 0.01$ ) for comparison of soil moisture effect, 15; for comparison of time of incubation, 22; and for interaction of soil moisture and time, 37.

of 0.94 and 1.88 mg N/g of soil, respectively, in both soil types (Fig. 3-A). After these sampling periods, however, sclerotium populations increased and reached levels above the controls at 7 weeks.

A statistical comparison showed that the effect of glucose and  $\text{NaNO}_3$  used at various C:N ratios was

greater than that of glucose alone. Sclerotium populations decreased by 58-61% of the control during the first week at all C:N ratios (Fig. 4-A). At C:N ratios of 10 and 20, the decline was slower than at 40 and 80, and continued to decline giving a reduction of 89-94% of the control, after 7 weeks in both soil types.

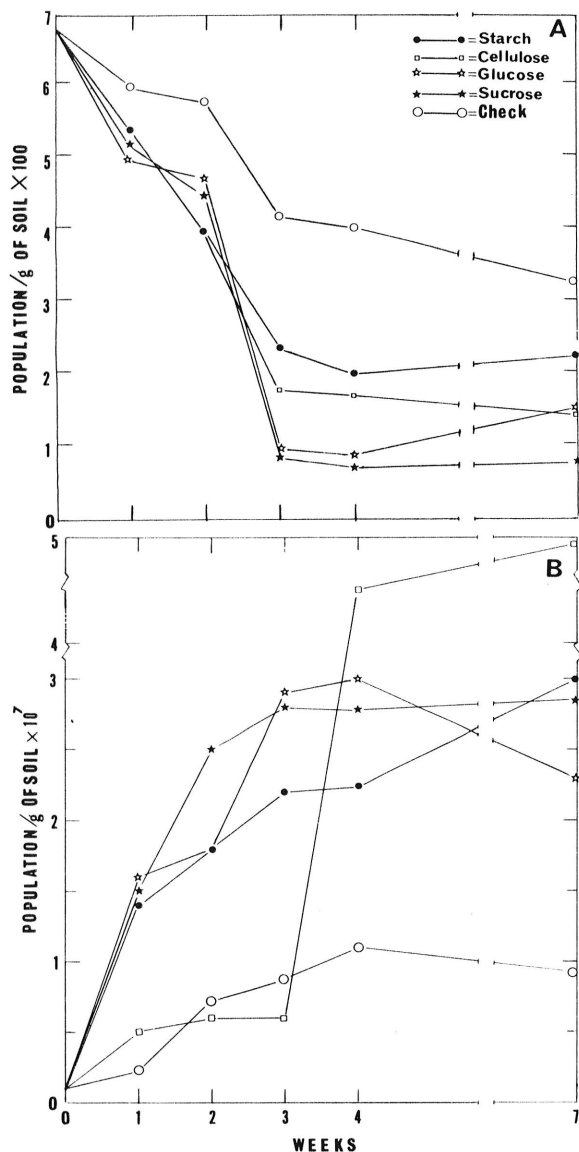


Fig. 2-(A, B). Changes in populations over 7 weeks in sandy-loam soil of: A) *Macrophomina phaseolina* and B) total microorganisms in soil nonamended or amended with different carbon sources. Data presented are means of two repetitions of three replications for each treatment. Correlation coefficients (r values) for decrease in *M. phaseolina* and increase in total microbial populations are: starch =  $-0.93^{**}$ ; cellulose =  $-0.72^{ns}$ ; glucose =  $-0.95^{**}$ ; sucrose =  $-0.87^{*}$ ; and control =  $-0.87^{*}$ , where  $^{**}$  = significant difference ( $P = 0.01$ ),  $^{*}$  = significant difference ( $P = 0.05$ ), and  $^{ns}$  = not significant. Fisher's LSD ( $P = 0.01$ ) for comparison of carbon sources, 34; for comparison of time of incubation, 16; and for interaction of carbon sources and time, 19.

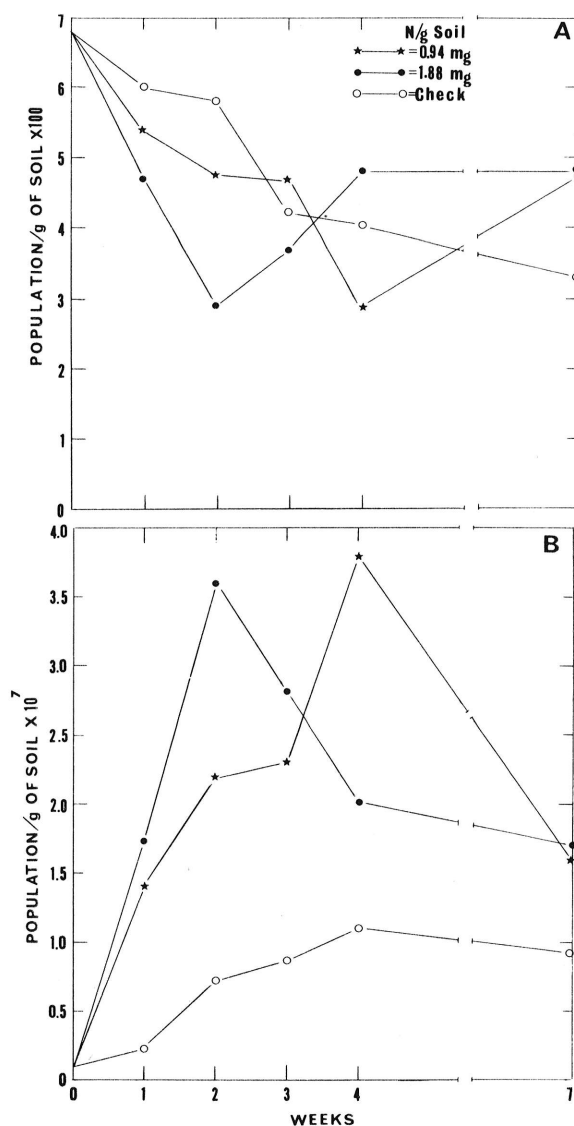


Fig. 3-(A, B). Changes in population over 7 weeks in sandy-loam soil of: A) *Macrophomina phaseolina* and B) total soil microbial populations nonamended (check) or amended with different nitrogen (N) concentrations. Data presented are means of two repetitions of three replications for each treatment. Correlation coefficients (r values) for decrease in *M. phaseolina* population and increase in total microbial population are for N at: 0.94 mg/g of soil =  $-0.99^{**}$ ; 1.88 mg/g of soil =  $0.99^{**}$ ; and control =  $-0.87^{ns}$ , where  $^{**}$  = significant ( $P = 0.01$ ) and  $^{ns}$  = not significant. Fisher's LSD ( $P = 0.01$ ) for the comparison of N levels, 19; for time of incubation, 11; and for interaction between N and time, 13.

In all amended soil samples there was an increase in total microbial populations over controls. There was a significant inverse correlation between changes in population of *M. phaseolina* and total microbial populations in both soil types (Fig. 2-B, 3-B, 4-B).

**DISCUSSION.**—The reduction in sclerotium populations of *M. phaseolina* and total fungi under high soil moisture can explain in part the reduction in charcoal rot recorded during a rainy period or by frequent irrigation (11, 17, 20, 27). The adverse effect of high soil moisture on some soil-borne pathogens was reported (5, 16, 26). Our in vitro tests (7) and those of Kavoor (19) suggest that the sclerotia of *M. phaseolina* may be directly parasitized by soil bacteria at high soil moistures. "Abnormal" germination of *M. phaseolina* sclerotia occurred in nonamended soils (1, 14, 30) and the germ tube and subsequent mycelium are very sensitive to bacterial attack at high soil moisture resulting in lysis and digestion of the fungal cell walls (19, 23).

In all amended soil samples, the sclerotium population of *M. phaseolina* was reduced below the controls. Sclerotia of *M. phaseolina* germinate normally in soils amended with sugars, amino acids, and root exudates (1, 14, 30). The reduction in *M. phaseolina* population was significantly correlated with increases in total microbial populations in both soil types. Our soil amendments, which caused a reduction in soil populations of sclerotia, may have stimulated germination, with the resulting germ tubes and hyphae being attacked by bacteria and actinomycetes (9, 19, 23). Although high concentration of CO<sub>2</sub> from increased microbial activity can temporarily check growth of *Rhizoctonia solani* (6, 25) and *Ophiobolus graminis* (8), it has no apparent effect on the viability of *M. phaseolina* sclerotia and mycelium (33).

A statistical comparison showed that the reduction of *M. phaseolina* sclerotium populations was greater in soils amended with amendments having C:N ratios of 10 and 20, than at 40 and 80, or in soils amended with glucose alone. There was no significant effect of nitrogen after 2 to 3 weeks. Nitrogen is rarely a limiting factor to microbial activity in the soil, except in the presence of high concentrations of available carbon; thus, the amount of nitrogen relative to the amount of carbon is of importance (16). A similar effect was reported on the chlamydospores of *Thielaviopsis basicola* (24) and microsclerotia of *Verticillium albo-atrum* (15). The increase in populations of *M. phaseolina* at 4 weeks in soils amended with starch, glucose, sodium nitrate alone, or at C:N ratios of 40 and 80 may be due to increased mycelial growth from germinating sclerotia and the subsequent formation of sclerotia. Meyer et al. (21) showed an increase in *M. phaseolina* populations in soils amended with soybean-stem-extracts after 7 days at 30 C.

Ghaffar et al. (13) controlled charcoal rot of cotton seedlings, whose roots were dipped in a mycelial suspension of *M. phaseolina*, by low C:N ratio organic soil amendments. They attributed the control to increases in populations of bacteria and actinomycetes antibiotic to *M. phaseolina* based on in vitro studies. They did not discuss the effect of amendments on inoculum density, which is directly related to disease severity, if all conditions are optimum for disease development (2), and did not take into account that mycelium of *M. phaseolina*

does not survive in soils for more than 7 days (9, 21).

A practical cultural control of charcoal rot could be developed by keeping soil moisture at 60% MHC for 2-3 weeks during a period where soil temperatures are about 30 C. In areas where ample water is not available, a combination of lower soil moisture and organic amendments might be used.

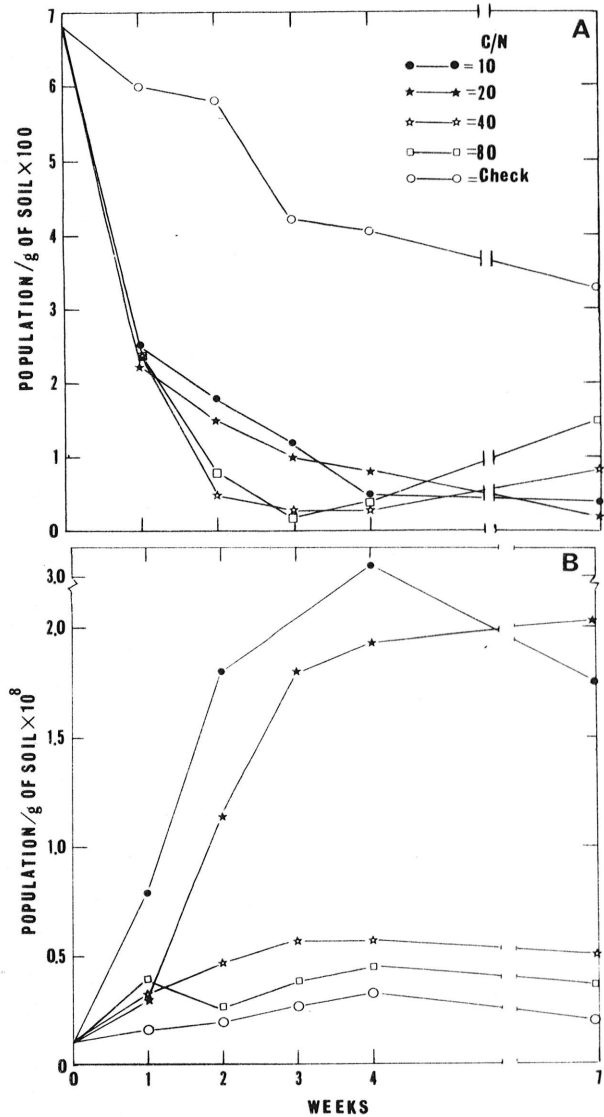


Fig. 4-(A, B). Changes in populations over 7 weeks in sandy-loam soil of A) *Macrophomina phaseolina* and B) total microbial populations nonamended (check) or amended with different carbon:nitrogen ratios. Data presented as means of two repetitions of three replications for each treatment. Correlation coefficients (*r* values) for decrease in *M. phaseolina* population and increase in total microbial population are for C:N ratios of: 10 = -0.85\*; 20 = -0.84\*; 40 = -0.91\*; 80 = -0.85; and control = -0.87\*, where \* = significance ( $P=0.05$ ). Fisher's LSD ( $P=0.01$ ) for comparison of C:N ratios, 18; for the time of incubation, 14; and for interaction of C:N ratios and time, 17; and for comparison with glucose and C:N ratios, 16; for time of incubation, 20; and for interaction of C:N ratio and time, 26.

## LITERATURE CITED

1. AYANRU, D. K. G., and R. J. GREEN, JR. 1974. Alteration of germination patterns of sclerotia of *Macrophomina phaseolina* on soil surface. *Phytopathology* 64:595-601.
2. BAKER, R. 1970. Use of population studies in research on plant pathogens in soil. *in*: Toussoun et al. (eds.). *Root diseases and soil borne pathogens*. University of California Press, Berkeley. 252 p.
3. CARMER, S. G., and M. R. SWANSON. 1971. Detection of differences between means: a Monte Carlo study of five pairwise multiple comparison procedures. *Agron. J.* 63:940-945.
4. COOK, G. E., M. G. BOOSALIS, L. D. DUNKLE and G. W. ODVODNY. 1973. Survival of *Macrophomina phaseoli* in corn and sorghum stalk residue. *Plant Dis. Rep.* 57:873-875.
5. COOK, R. J., and R. I. PAPENDICK. 1970. Soil water potential as a factor in the ecology of *Fusarium roseum* f. sp. *cerealis* 'Culmorum'. *Plant Soil.* 32:131-145.
6. DAVEY, C. B., and G. C. PAPAIVIZAS. 1963. Saprophytic activity *Rhizoctonia* as affected by carbon-nitrogen balance of certain organic amendments. *Soil Sci. Soc. Am. Proc.* 27:164-167.
7. DHINGRA, O. D. 1974. Saprophytic survival of *Macrophomina phaseolina* in soil. Ph.D. thesis, University of Illinois, Urbana, 58 p.
8. GARRETT, S. D. 1970. *Pathogenic root-infecting fungi*. Cambridge University Press, Cambridge, England. 294 p.
9. GHAFAR, A. 1968. Survival of *Macrophomina phaseoli* (Maubl.) Ashby, the cause of root rot of cotton. *Pak. J. Sci. Res.* 20:112-118.
10. GHAFAR, A., and P. AKHTAR. 1968. Survival of *Macrophomina phaseoli* (Maubl.) Ashby on cucurbit roots. *Mycopathol. Mycol. Appl.* 35:245-248.
11. GHAFAR, A., and D. C. ERWIN. 1969. Effect of soil water stress on root rot of cotton caused by *Macrophomina phaseoli*. *Phytopathology* 59:795-797.
12. GHAFAR, A., and G. PARVEEN. 1969. Colonization of cotton roots by soil micro-organisms. *Mycopathol. Mycol. Appl.* 38:373-376.
13. GHAFAR, A., G. A. ZENTMYER, and D. C. ERWIN. 1969. Effect of organic amendments on the severity of *Macrophomina* root rot of cotton. *Phytopathology* 59:1267-1269.
14. GREEN, R. J., JR., and D. K. G. AYANRU. 1973. Effect of soil fungistasis on germination patterns of sclerotia of *Macrophomina phaseoli*. Abstract 0034 *in* Abstracts of papers, 2nd Int. Congr. Plant Pathol., 5-12 September, Minneapolis, Minnesota (unpaged).
15. GREEN, R. J., JR., and G. C. PAPAIVIZAS. 1968. The effect of carbon sources carbon to nitrogen ratios, and organic amendments on survival of propagules of *Verticillium albo-atrum* in soil. *Phytopathology* 58:567-570.
16. GRIFFIN, D. M. 1972. *Ecology of soil fungi*. Syracuse University Press, Syracuse, New York. 193 p.
17. HODGES, C. S. 1962. Black root rot of pine seedlings. *Phytopathology* 52:210-219.
18. ILYAS, M. B., and J. B. SINCLAIR. 1974. Effects of plant age upon the development of necrosis and occurrence of intraxylem sclerotia in soybean infected with *Macrophomina phaseolina*. *Phytopathology* 64:156-157.
19. KAVOOR, A. T. A. 1954. Some factors affecting the growth of *Rhizoctonia bataticola* in soil. *J. Madras Univ.* 24:47-52.
20. LIVINGSTON, J. E. 1945. Charcoal rot of corn and sorghum. *Nebr. Agric. Exp. Stn. Res. Bull.* 136. 32 p.
21. MEYER, W. A., J. B. SINCLAIR, and M. N. KHARE. 1973. Biology of *Macrophomina phaseoli* in soil studied with selective media. *Phytopathology* 63:613-620.
22. MEYER, W. A., J. B. SINCLAIR, and M. N. KHARE. 1974. Factors affecting charcoal rot of soybean seedlings. *Phytopathology* 64:845-849.
23. NORTON, D. C. 1953. Linear growth of *Sclerotium bataticola* through soil. *Phytopathology* 43:633-636.
24. PAPAIVIZAS, G. C. 1968. Survival of root infecting fungi in soil. VI. Effect of amendments on bean root rot caused by *Thielaviopsis basicola* and on inoculum density of the causal organism. *Phytopathology* 58:421-428.
25. PAPAIVIZAS, G. C., and C. B. DAVEY. 1962. Activity of *Rhizoctonia* in soil as affected by carbon dioxide. *Phytopathology* 52:759-766.
26. PAPAIVIZAS, G. C., and J. A. LEWIS. 1971. Survival of endoconidia and chlamydospores of *Thielaviopsis basicola* as affected by soil environmental factors. *Phytopathology* 61:108-113.
27. PRASAD, N. 1944. Studies on the root rot of cotton in Sind, I. *Indian J. Agric. Sci.* 12:40-43.
28. RADHA, K. 1956. Soil conditions and root diseases. XVI. Colonization and survival of *Macrophomina phaseoli* (Maubl.) Ashby in trace element amended soils. *J. Indian Bot. Soc.* 33:47-52.
29. REICHERT, I., and E. HELLINGER. 1947. On the occurrence, morphology and parasitism of *Sclerotium bataticola*. *Palestine J. Bot.* 6:107-147.
30. SMITH, W. H. 1969. Germination of *Macrophomina phaseoli* sclerotia as affected by *Pinus lambertiana* root exudate. *Can. J. Microbiol.* 15:1387-1391.
31. SMITH, W. H. 1969. Comparison of mycelial and sclerotial inoculum of *Macrophomina phaseoli* in the mortality of pine seedlings under varying soil conditions. *Phytopathology* 59:379-382.
32. TUIE, J. 1961. *Plant pathological methods*. Burgess, Minneapolis, Minnesota. 239 p.
33. VASUDEVA, R. A. 1937. Studies on the root rot disease of cotton in Punjab. III. The effect of some physical and chemical factors on sclerotia formation. *Indian J. Agric. Sci.* 7:259-270.
34. WARCUP, J. H. 1950. The soil plate method for isolation of fungi from soil. *Nature* 166:117-118.
35. ZACHARIAH, A. T. 1953. Soil conditions and root diseases. IX. Fungal ecology of cultivated fields. *Proc. Indian Acad. Sci., B* 38:235-241.