Research Bulletin No. 37 November 1987

INTER AND INTRA ANTAGONISTIC EFFECTS OF SOIL-BORNE ORGANISMS UPON SOYBEANS

By Charles Cottingham
Yvonne D. Riley
and
Ukeme E. Eyo

Published as a Technical Contribution From

AFS
South Carolina State College
Orangeburg, South Carolina

In Cooperation With Cooperative State Research Service, USDA

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

JUSTIFICATION 1

RELATED LITERATURE 2

ABSTRACT 3

INTRODUCTION 4

OBJECTIVES 4

MATERIALS AND METHODS 5

RESULTS AND DISCUSSION

A. Height and Dry-weight of Bragg (Untreated) Trials I 7

B. Height and Dry-weight of Bragg (Untreated) Trials II 9

C. Height and Dry-weight of Coker 237 (treated and untreated) Trials I 11

D. Height and Dry-weight of Coker 237 (treated and untreated) Trials II 14

E. Height and Dry-weight of Coker 237 (untreated) Trials I 16

F. Height and Dry-weight of Coker 237 (untreated) Trials II 18

G. Height and Dry-weight of Braxton (untreated) Trials I 20

H. Height and Dry-weight of Braxton (untreated) Trials II 22

EFFECTS OF **HOPLOLAIMUS COLUMBUS** ON **MACROPHOMINA PHASEOLINA** IN SOYBEAN 26

LITERATURE CITED 27

ACKNOWLEDGEMENTS

The authors, Dr. Charles Cottingham, Mrs. Yvonne D. Riley, and Mr. Ukeme E. Eyo[1], wish to express their thanks to the Cooperative State Research Service (CSRS), United States Department of Agriculture (USDA) for funding this research under Public Law 95-113, and to Dr. Robert L. Hurst, Vice President for Research, Planning and Extension; Dr. James E. Walker, Jr., Senior Associate Research Director; Dr. Lewie C. Roache, Dean of the School of Arts and Sciences; and Dr. James H. Arrington, Chariman of the Department of Natural Sciences, South Carolina State College for their timely advice, encouragement, and cooperation. A special note of gratitude is extended to Dr. Hill, Superintendent of the Edisto Experiment Station, and Dr. John Mueller for their cooperation on the nematode phase of this study.

A final note of gratitude is extended to the 1890 Clerical Center and student assistants for their participation in this research endeavor.

To all others who contributed to this study, but go unmentioned, the authors are deeply grateful.

---

[1]Professor, Electron Microscopist, and Technician, respectively, all of the Department of Natural Sciences, South Carolina State College, Orangeburg, SC 29117.

INTER AND INTRA ANTAGONISTIC EFFECTS OF SOIL-BORNE ORGANISMS UPON SOYBEANS

Justification

Soybean ranks first as a cash crop in South Carolina. Increased acreage of the crop in recent years has decreased crop rotation, thus, creating a condition whereby soybeans are often planted for two or more years on the same land, either without rotation or in a one year rotation with small grains/or corn. Under these conditions, a gradual build-up of soil-borne pathogens which attack soybean can be expected. Simultaneously, a significant decrease in yield can also be expected.

That antagonism exists between soil-borne organisms was demonstrated by Pettit **et al.** (39) when they isolated a strain of **Bacillus subtilis** from interiors of peanut cotyledons that produced an antibiotic which affected the growth of species of **Macrophomina, Choanephora, Chaetomium,** and **Penicillium.** Dennis and Webster (14) identified acetaldehyde as a volatile inhibitor produced by **Trichoderma** spp. when assayed in vitro against several soil-borne pathogens. Wells **et al.** (46) reported that the disease caused by **Sclerotium rolfsii** was reduced when **Trichoderma harzianum** was used as a biological amendment to field soil.

While a voluminous amount of work has been done on damage caused by seed and soil-borne root-infecting fungi on tomatoes (17), (30), wheat (8), (21), (24), cotton (1), (2), (22), and tobacco (4), 16), (23), there exists only sparse information on research efforts focusing on the relationship between soil-borne organisms and **Glycine max** with emphasis on biological control.

This research proposes to isolate soil-borne fungi from soybean roots grown in the field, identify species isolated, grow cultures of the isolates in the laboratory to show any antagonistic characteristics, and set up a complete randomized block experiment in the greenhouse to demonstrate the effects each organism has upon soybean singularly, and in combination.

RELATED LITERATURE

The soybean, **Glycine Max.** (L.) Merrill. is a native of eastern Asia. Its early history is lost in antiquity. The first record of the plant in China dates back to 2838 B.C. (35). It was one of the five sacred grains upon which Chinese civilization depended.

Expanded soybean acreage has accounted for an increase in numbers of diseases and their severity due to inoculum build-up when rotation was unfeasible. This is a detrimental factor when the vast acreage of an economic crop which produces billions of dollars in income is threatened.

Control measures become important when we realize that more than 100 pathogens (soil-borne, seed-borne and wind-blown) are constantly on the attack. All parts of the plant are susceptible to a host of pathogens which reduce yield and seed quality, thus, oil quality and quantity.

Through all this dismal smoke-screen we can see a ray of hope offered by the very antagonistic behavior of micro-organisms to each other.

Volatile metabolites of microbial activity released into soil play an important role in the phenomenon of fungistasis (5, 14, 26, 27, 41, 44, 45, 47). Fungistatic nature of organisms allows for some control by impeding the growth of other organisms, but death does not ensue. Fungicidal activity is the desired mechanism, but is difficult to discover. Hence, this is a major thrust of this research effort.

Trichoderma spp. are widespread in the soils of South Carolina. It was reported by Daines (13) to produce antibiotic activity detrimental to the growth of **Streptomyces scabies.** The growth of **Sclerotium rolfsii** was inhibited by **Trichoderma** spp. in culture, and the severity of sclerotial blight was reduced in soybean on greenhouse tests (36). Wells **et al.** (46) used **Trichoderma harzianum** as a biological amendment to field soil to reduce the incidence of **Sclerotium rolfsii.** It exhibited some biological control, but the mechanism involved was not given. Kilpatrick and Johnson (28), (29) isolated **Trichoderma** spp. from seed still within pods. This indicated that the organism was an internal parasite that grew through the soybean plant from its soil-borne habitat. This lends credence to objective number 2. Singh **et al.** (43) reported that **Trichoderma** spp. were found on soybean seeds.

Macrophomina phaseolina is the other fungus that will be used to show if any fungistatic/or fungicidal properties exists between it and **Trichoderma** spp. when grown with soybean host plants.

Pettit **et al.** (39) isolated a strain of **Bacillus subtilis** from interiors of peanut cotyledons antagonistic to species of **Macrophomina, Chaonephora, Chaetomium,**

and Pencillium. Dennis and Webster (14) reported that volatile inhibitors were produced by **Trichoderma** spp. when assayed in vitro against several soil-borne pathogens. **M. phaseolina** was one of those pathogens identified. Acetaldehyde was reported to have sporastatic properties in its vapor phase on several species of fungi by Robinson and Park (40). Sclerotia of two specimen of **M. phaseolina** were found to occur in vessels and pith of lower parts of soybean stems in Greece (10). This indicated that the fungus is an internal parasite. It further supports the significance of objective number 2.

ABSTRACT

Charcoal rot of soybeans (Glycine max), caused by **Macrophomina phaseolina,** reduced height and dry-weight of infected plants. We evaluated the hyperparasitic properties of **Trichoderma harzianum** on **M. phaseolina** by applying **T. harzianum** in a wheat-bran mixture (800,000 conidia/g.) and **M. phaseolina** in an aqueous suspension (650,000 sclerotia/ml.) to seed of soybean cultivars Bragg, Coker 237, and Braxton before, and at planting. The seed were either treated with captan (cis-N-[(trichloromethyl)]-4cyclohexene-1, 2-dicarboximide) at 1.6g/kg of seed, or were left untreated. Addition of the pathogen and antagonist in combination suppressed the pathogen more in plants grown from treated than untreated Coker 237 seed. Plants grown from **M. phaseolina** -inoculated Coker 237 seed that were treated with fungicide or left untreated were shorter than plants from seed receiving both fungi or **T. harzianum** alone. Plants from Coker 237 seed receiving **M. phaseolina** alone, or **T. harzianum** had lower dry-weight than controls regardless of fungicide treatment.

The expected pattern of suppression; pathogen > fungal combination > antagonist with respect to shoot height, shoot dry-weight, and root dry-weight, did not develop in trials with the three soybean cultivars. The antagonist suppressed growth more than the pathogen or fungal combination in most trials. The antagonist increased dry-weight in Coker 237 in treated vs untreated trials.

Ultrastructural investigations revealed that **M. phaseolina** penetrated epidermal cells directly. Hyphae grew intercellularly, intracellularly, vertically and laterally throughout cortical parenchyma and xylem vessels by direct penetration of cell walls or through pits. Small rounded protrusions that developed on hyphae appeared to be terminal and intercalary chlamdospores or microsclerotia produced during reproduction.

INTRODUCTION

Interactions among **Macrophomina phaseolina** (Tassi) Goid., and **Trichoderma harzianum** Rifai, although of major importance to those interested in increasing soybean (Glycine max [L.] merr.) production and enhancing biological control, have not been studied concurrently. Although both micro-organisms frequently occur simultaneously in the roots or rhizosphere of the same soybean plant, their combined effects have not been investigated.

Elad et al (18) found that incorporation of the wheat-bran inoculum preparation of **T. harzianum** in pathogen-infested soil significantly reduced bean diseases caused by **Sclerotium rolsfii,** and **Rhizoctonia solani** Kuehn. In a more recent study, they reported a 70% disease reduction of **R. solani** in carnation with the wheat-bran culture of **T. harzianum** (19). Antagonistic properties of **T. lignorum** (Tode) Harz. on **R. solani** were reported as early as 1934 (14).

Interaction between **M. phaseolina** and the test organism could not be found in the literature, which prompted this study. The purpose of this investigation is to determine the effects of the antagonist, **T. harzianum** on the charcoal rot fungus, **M. phaseolina** in soybean.

OBJECTIVES

1. To isolate and identify soil-borne fungi and Lance nematodes from soybean roots. If isolation should fail, cultures of **Trichoderma, Macrophomina,** and nematodes will be purchased from Type Culture Laboratory.
2. To establish a pattern of entry and growth for each organism with relationship to all others through scanning electron microscopy.
3. To show any antagonistic/or stimulatory effects these organisms may possess between each other, and with their soybean host.

MATERIALS AND METHODS

Plant and soil materials. Seeds of soybean cultivars Coker 237 and Bragg, were kindly supplied by, The Dublin Seed Company, Orangeburg, South Carolina [illegible]5. These cultivars of maturity groups VI and VII were grown in a greenhouse at Orangeburg. Plastic pots 20 cm in diameter, fitted with saucers, were filled with an autoclaved sandy clay loam soil with an organic matter content of 34.4 g/kg of soil (3). One seed of each cultivar was planted in each experimental pot. The experimental designs were a randomized complete block with varying numbers of replications and treatments.

Fungal inoculum. **M. phaseolina** was propagated in a greenhouse on 'Bragg' soybean from an isolate obtained from the Edisto Branch of the South Carolina Agricultural Experiment Station at Blackville. Roots were air-dried and ground in a Wiley Mill fitted with a (40-mesh) screen. A 1 g sample of this inoculum was plated on a selective medium consisting of chloroneb-mercuric chloride rose bengal agar (CMRA) developed by Meyer **et al.** (34), and modified by Cottingham (12). Samples were plated on the modified medium (MCMRA) by using the microsmound technique of Bristow and Wyllie (6). Inoculated plates were incubated at 30c until the agar was dry. Sclerotia were scraped from the agar with a spatula and stored in sealed vials. Two mg of this inoculum produced 7 x 10^5 sclerotia by standard hemacytometer counts of 5 fields in 50 ml of distilled water. Five ml (7×10^4 sclerotia) were added to each hole in each experimental pot. Medium for growing inoculum of **T. harzianum** was prepared (25) by autoclaving a wheat-bran and tap water mixture (1:2 v/v) for 1 hour a day at 121 C on two successive days. Flasks containing this medium were inoculated with **T. harzianum,** incubated for 3 days at 30 c, illuminated for 12 hours and then incubated for 5 days. Fungal preparations contained (6 x 10^6 conidia)/g dry weight by standard hemocytometer counts. A .lg sample of inoculum (6 x 10^5 conidial) was added to each experimental pot.

SEM procedures. Roots from soil amended with **M. phaseolina** and **T. harzianum** were washed in distilled water, dried, and sectioned into 5-8mm pieces. Each piece was split longitudinally, and secured to a 15 mm H x 15 mm aluminum specimen mount (both sides face-up) with conducting paint (11). Sections were coated with gold 150-200A°, in a Denton Vacuum (DV-515) automatic evaporator (Denton Vacuum, Incorporated, Cherry Hill, N. J. 08003) and viewed in a super II scanning electron microscope (International Scientific Instruments, Incorporated, Santa Clara, CA 95050).

Data collection and analysis. When soybeans reached maturity, shoot height (centimeters), shoot and root dry weight (grams), and percent disease/antagonism/biological control by the antagonist, **(T. harzianum)**, were determined. Shoot and root growth were the mean height and dry weight, respectively, of one or more plants per three or more replications per treatment. Experiments were repeated two or more times and data were combined. The mean number of plants per treatment varied.

RESULTS AND DISCUSSION

Height and Dry-Weight of Bragg (untreated) Trials I

Height of soybeans grown in soil amended with **T. harzianum** and **M. phaseolina** applied two weeks prior and at planting, respectively, were significantly reduced. The reduced growth indicates a parasitic pathogen/host relationship. **T. harzianum** appears to be parasitic when amended to soil two weeks prior to planting, however, when applied at planting time or with **M. phaseolina** prior to and at planting, did not significantly reduce growth as did **M. phaseolina** at planting time (Table 1). These data support the findings of Kilpatrick and Johnson (29) that **T. harzianum** may act as a parasite.

TABLE 1. Height in cm of 45-day Old Bragg Plants grown in soil amended with **Macrophomina phaseolina** and **Trichoderma harzianum** two-weeks before and at planting.

Treatment[a]	Replicates 1	2	3	4	5	Average Total
1	29.9	33.0	38.9	37.0	33.1	34.46 ab
2	41.8	40.4	36.7	35.1	34.3	37.66 bc
3	38.9	43.9	36.2	39.0	37.5	39.10 c
4	36.7	40.1	39.4	31.8	30.9	34.78 ab
5	36.3	38.5	40.4	41.9	36.7	38.76 c
6	39.1	36.0	42.9	40.1	36.5	38.92 c
ck	41.3	44.0	38.4	40.9	38.6	40.46 c

[a] T1 - **T. harzianum** applied 2 weeks before planting
T2 - **T. harzianum** applied at planting
T3 - **M. phaseolina** applied 2 weeks before planting
T4 - **M. phaseolina** applied at planting
T5 - **T. harzianum + M. phaseolina** applied 2 weeks before planting
T6 - **T. harzianum + M. phaseolina** applied at planting
T7 - Uninoculated check

Column values followed by the same letter are not significantly different (P = 0.05) using Duncan's Multiple range test.

The dry-weight of soybeans was significantly reduced when T [illegible] **M. phaseolina** and both combined were applied, at planting time. No significant reduction occurred when the antagonist, pathogen and both combined were applied two weeks prior to planting time (Table 2).

TABLE 2. Dry-weight of 45-day old Bragg Plants grown in soil amended with **Macrophomina phaseolina** and **Trichoderma** 2-weeks before and at planting

Treatments[a]	Replicates 1	2	3	4	5	Average Total
1	3.4	3.5	4.5	4.5	3.0	3.78 cd
2	2.6	1.8	1.83	1.33	0.67	1.64 a
3	4.5	4.0	2.3	3.0	2.6	3.28 bcd
4	2.7	3.4	2.0	2.1	2.3	2.50 ab
5	3.0	2.7	3.4	3.5	3.2	2.16 bcd
6	2.7	3.0	3.8	3.0	1.5	2.80 bc
ck	3.5	4.0	4.8	4.6	3.2	4.02 d

[a]T1 - **T. harzianum** applied 2 weeks before planting
T2 - **T. harzianum** applied at planting
T3 - **M. phaseolina** applied 2 weeks before planting
T4 - **M. phaseolina** applied at planting
T5 - **T. harzianum + M. phaseolina** applied 2 weeks before planting
T6 - **T. harzianum + M. phaseolina** applied at planting
T7 - Uninoculated check

Column values followed by the same letter are not significantly different (P = 0.05) using Duncan's Multiple range test.

RESULTS AND DISCUSSION

Height and Dry-weight of Bragg (untreated) Trials II

Effect of Pathogen/Antagonist on Shoot Height. Shoot height was reduced .6% in fungal combination relative to the control, while a 10.2% and an 8.7% reduction occurred, respectively, with reference to the pathogen and the antagonist (Table 1).

A 9.7% reduction in height occurred in pathogen only treatments compared with fungal combinations, while an 8.2% reduction occurred when combinations were compared with the antagonist only. The pathogen only, reduced shoot height 1.7% relative to the antagonist (Table 1).

TABLE 1. Mean disease severity of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with the antagonist **Trichoderma harzianum**

Cultivar	Treatment	Shoot Height[a] (cm)	Reduction/Increase[b] %
Bragg	Mp + TH	141.0	0.56
	Mp Only	127.3	10.23
	Th Only	129.5	8.67
	Control	141.8	- -

[a]Each value represents the mean shoot height of 4 experiments with 5 replicates, and 1 plant/replicate.
[b]Each value represents the percent increase or decrease of shoot height relative to controls.

Effect of Pathogen/Antagonist on Shoot Dry-Weight. Shoot dry-weight increased 7.2% in fungal combination relative to the control. In contrast, the pathogen only, and the antagonist only, reduced dry-weight 3.3%, and 21.6% respectively (Table 2).

A 9.8% reduction in shoot dry-weight occurred in pathogen only trials with reference to fungal combinations, compared with a 26.8% reduction in antagonist only trials. The antagonist only reduced dry-weight 18.6% relative to pathogen only trials (Table 2).

TABLE 2. Mean disease severity of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with **Trichoderm harzianum**

Cultivar	Treatments	Dry Weight[a] (g)	Reduction/Increase[b] %
Bragg	Mp + Th	19.4	7.18
	Mp Only	17.5	3.31
	Th Only	14.2	21.55
	Control	18.1	- -

[a]Each value represents the mean shoot dry-weight of 4 experiments with 5 replicates, and 1 plant/replicate.

[b]Each value represents the percent decrease or increase of shoot dry weight relative to controls.

Effect of Pathogen/Antagonist on Root Dry-Weight. Fungal combinations reduced root dry-weight 16.2% relative to controls, while the pathogen only, and the antagonist only, reduced dry-weight 24.3%. A 9.7% reduction occurred in trials where both pathogen, and antagonist were compared to fungal combinations (Table 3).

TABLE 3. Mean disease severity of charcoal rot caused by **Macrophomina phasolina** on soybean in soil amended with **Trichoderma harzianum**

Cultivar	Treatments	Root dry-weight[a] (g)	Reduction/Increase[b]
Bragg	Mp + Th	3.1	16.22
	Mp Only	2.8	24.32
	Th Only	2.8	24.32
	Control	3.7	- -

[a]Each value represents the mean root dry-weight of 4 experiments with 5 replicates, and 1 plant/replicate.

[b]Each value represents the percent decrease in root dry-weight relative to the control.

RESULTS AND DISCUSSION

Height and Dry-Weight of Coker 237 (treated and untreated) Trials I

Effect of Pathogen/Antagonist on Shoot Height. Shoot height was reduced only 3% below the control in trials where the pathogen only was applied to soil in which treated Coker 237 seeds were planted, compared with an 8.8% increase in untreated Coker 237 trials relative to controls (Table 1). Fungal combinations produced an increase of 2.17% over the control in trials with treated Coker 237 seed. In contrast, a 4.8% decrease relative to the control was noted in untreated Coker 237 trials. The antagonist only, reduced shoot height 18.6%, and 7.2 respectively, in treated and untreated Coker 237 trials. Within Coker 237 trials, the pathogen alone reduced shoot height 9.3% compared to fungal combinations, while height was reduced 23.8% by the antagonist alone over fungal combinations. A 16% reduction in shoot height was produced by the antagonist relative to the pathogen. Within untreated Coker 237 trials, the pathogen alone reduced shoot height 4.2% compared to fungal combinations, while a 2.5% reduction was produced by the antagonist alone. A 1.8% decrease in shoot height was produced by the pathogen relative to the antagonist.

TABLE 1. Mean disease severity of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with the antagonist **Trichoderma harzianum**

Cultivar	Treatments	Shoot height[a] (cm)	Increase/decrease[b] %
Coker 237 (treated)	Mp + Th	117.5	+107.73
	Mp Only	106.6	3.00
	Th Only	89.5	18.56
	Control	109.9	---
Coker 237 untreated)	Mp + Th	97.2	4.80
	Mp Only	93.1	8.81
	Th Only	94.8	7.15
	Control	102.1	---

[a]Each value represents the mean shoot height of 4 experiments with 5 replicates, and 1 plant/replicate.

[b]Each value represents the percent increase or decrease of shoot height relative to control.

Effect of Pathogen/Antagonist on Shoot Dry Weight. Dry-weight of shoots from treated Coker 237 soybean seeds grown in soil amended with the antagonist, **T. harzianum** and the pathogen **M. phaseolina** were not significantly reduced relative to the control (Table 2). A 12.4% dry-weight reduction occurred in pathogen only trials relative to the control, while an 8.5% dry-weight increase over the control occurred in antagonist only trials. No significant difference occurred when fungal combinations were compared with the control in untreated Coker 237 trials (Table 2). However, a 17.5% and a 21.7% dry-weight reduction, respectively, occurred in pathogen only, and antagonist only trials.

Comparisons between fungal combinations in treated and untreated Coker 237 trials produced a 25.5% dry-weight reduction in the former. In pathogen only trials, a 17.5% reduction occurred in the treated vs untreated group. In contrast, the antagonist only, produced a 7.1% dry-weight increase in the treated vs untreated group. This increase in dry-weight by **T. harzianum** is in agreement with data reported by Y. Chun Chang et al. (9).

TABLE 2. Mean disease severity of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with the antagonist **Trichoderma harzianum**

Cultivar	Treatments	Shoot dry weight[a] (g)	Reduction/increase[b] %
Coker 237 (treated)	Mp + Th	12.0	6.98
	Mp Only	11.3	12.40
	Th Only	14.0	+8.53
	Control	12.9	--
Coker (untreated)	Mp + Th	16.1	1.81
	Mp Only	13.7	17.47
	Th Only	13.0	21.69
	Control	16.6	---

[a]Each value represents the mean shoot dry weight of 4 experiments with 5 replicates, and 1 plant/replicate.

[b]Each value represents the percent reduction or increase of dry weight relative to controls.

Effect of Pathogen/Antagonist on Root Dry-Weight. Dry-weight was reduced 20.4% relative to the control in treated Coker 237 using fungal combination (Table 3). In contrast, a 9.1% increase resulted in similar combinations when untreated Coker 237 was used. In trials using the pathogen only with treated seeds, a 55.8% increase in dry weight resulted, while a 39.4% dry-weight increase was produced in untreated trials. The antagonist produced a 24.1% increase in root dry-weight in treated seed trials, while a 24.2% increase occurred in untreated trials relative to controls.

Within treated Coker 237 trials, the pathogen reduced dry-weight 44.2% relative to fungal combinations, and 41.5% relative to the antagonist. The antagonist only, reduced dry-weight 4.7% relative to fungal combinations (Table 3).

Within untreated Coker 237 trials, the pathogen reduced dry-weight 44.4% relative to fungal combinations, and 20% relative to the antagonist. The antagonist only, reduced dry-weight 30.6% relative to fungal combinations (Table 3).

TABLE 3. Mean disease severity of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with the antagonist **Trichoderma harzianum**

Cultivar	Treatments	Root dry weight[a] (g)	Reduction/Increase[b] %
Coker 237 (treated)	Mp + Th	4.3	20.37
	Mp Only	2.4	55.56
	Th Only	4.1	24.07
	Control	5.4	--
Coker 237 (untreated)	Mp + Th	3.6	+9.09
	Mp Only	20.0	39.39
	Th Only	2.5	24.24
	Control	3.3	--

[a]Each value represents the mean root dry weight of 4 experiments with 5 replicates, and 1 plant/replicate.

[b]Each value represents the percent reduction or increase of root dry weight relative to controls.

RESULTS AND DISCUSSION

Height and Dry-weight of Coker 237 (treated and untreated) Trials II

Effect of Pathogen/Antagonist on Shoot Height. Shoot height was reduced 19.2% by the pathogen only, relative to the control, while a 13.4 and a 13.2% reduction occurred in fungal combinations, and the antagonist, respectively, in treated seed trials (Table 1). There appeared to be no significant differences between treatments, thus, no analyses were run. The antagonist reduced shoot height on an equal basis with fungal combinations, suggesting that some form of metabolite might be involved.

In untreated trials, a 7.6% shoot height reduction occurred in the pathogen relative to the control, while a 6.8 and a 5.5% reduction occurred, respectively, in the antagonist and fungal combinations. The antagonist alone, had a more devastating effect on the host, than fungal combinations. This, again, indicates that something in the antagonist is causing it to act as a pathogen (45).

TABLE 1. Mean plant height in cm of mature soybean plants inoculated with **Macrophomina phaseolina** and **Trichoderma harzianum**

Cultivar	Treatments	Shoot height[a] (cm)	Reduction/Increase[b] %
Coker 237 (treated)	Mp Only	103.4	19.2
	Mp + Th	110.9	13.4
	Th Only	111.1	13.2
	Control	128.0	--
Coker 237 (untreated)	Mp Only	95.1	7.6
	Th Only	95.9	6.8
	Mp + Th	97.2	5.5
	Control	102.9	--

[a]Each value represents the mean shoot height of 4 experiments with 5 replicates, and 2 plants/replicate.

[b]Each value represents the percent decrease or increase of shoot height relative to controls.

Effect of Pathogen/Antagonist on Shoot Dry-Weight. The comparison between treated and untreated Coker 237 seed trials produced no significant reduction in shoot dry-weight when controls were compared with the pathogen only (Table 2). However, a 19% reduction occurred in non-treated fungal combinations compared with a 4% reduction in treated trials. The antagonist only reduced shoot dry-weight 49.1% and 26.6% respectively, in treated and untreated trials.

The expected pattern of shoot dry-weight reduction was pathogen > fungal combinations > antagonist developed with the former two, but not in the latter. The antagonist reduced shoot dry-weight 48.9% more than the pathogen, and 91.9% more than fungal combinations in treated trials, while a 10.2%, and a 25.9% reduction, respectively, were noted for pathogen and fungal combinations in untreated trials.

TABLE 2. Mean plant dry-weight in grams of mature soybean plants inoculated with **Macrophomina phaseolina** and **Trichoderma harzianum**

Cultivar	Treatments	Shoot dry-weight[a] (g)	Decrease/Increase[b] %
Coker 237 (treated)	Mp + Th	16.8	4.0
	Mp Only	13.1	25.1
	Th Only	8.9	49.1
	Control	17.5	--
Coker 237 (untreated)	Mp + Th	15.1	19.7
	Mp Only	14.3	23.9
	Th Only	13.8	26.6
	Control	18.8	--

[a]Each value represents the mean shoot dry-weight of 4 experiments with 5 replicates, and 2 plants/replicate.

[b]Each value represents the percent decrease or increase of shoot dry-weight relative to controls.

RESULTS AND DISCUSSION

Height and Dry-weight of Coker 237 (untreated) Trials I

Effect of Pathogen/Antagonist on Shoot Height. The height of soybean plants grown in soil amended with **T. harzianum** and **M. phaseolina** applied at planting was significantly reduced. The reduced growth indicates an antagonist/pathogen/host relationship (Table 1). Height of soybeans increased 9.5% over controls when soils were amended with **T. harzianum** alone, while height was reduced 11.7% in soils amended with **M. phaseolina** alone. Height was reduced 9.1% in plants grown in soil amended with **M. phaseolina** over plants where fungal combinations were applied. Plant height increased 11.2% in soil amended with **T. harzianum** only, over those in combinations of antagonist and pathogen, and 8.7% over controls. Disease/antagonism incidence was expressed as a percentage of increase or decrease of growth relative to controls.

The expected pattern of reduction in shoot height of the host should be in the order of pathogen > fungal combinations > antagonist. This order developed with respect to the pathogen, but not with fungal combinations and the antagonist. That **Trichoderma** spp. behave as a parasite was demonstrated by Kilpatrick and Johnson (29) who isolated the organism from soybean seed still in the pod. **T. harzianum** has also been shown to increase growth of various floricultural and horticultural crops (9). Thus, the apparent erratic behavior of this organism is not fully understood. However, it appears to be a facultative saprophyte that possesses volatile inhibitors as described by Dennis and Webster (14).

TABLE 1. Mean plant height of Coker 237 soybean plants grown in soil amended with **Macrophomina phaseolina** and **Trichoderma harzianum** singularly and in combination

Treatments	Shoot height[a] (cm)	Decrease/Increase[b] %
Mp + Th	65.6	2.81
Mp Only	59.6	11.70
Th Only	73.9	+9.48
Control	67.5	--

[a]Each value represents the mean height of 7 experiments with 3 replicates each, and 8 plants/replicate.

[b]Each value represents the percent increase or decrease relative to control.

Effect of Pathogen/Antagonist on Shoot Dry-Weight. Dry-weight of Shoots grown in soil amended with the antagonist and the pathogen applied at planting was significantly reduced relative to controls. (Table 2). The antagonist in conjunction with the pathogen alone reduced dry weight more than 19%. In contrast, the antagonist alone increased dry weight approximately 16.%. Treatment with the pathogen only, reduced dry weight 13.2% relative to pathogen/antagonist treatments, however, treatment with the antagonist only, increased dry weight 19.7%.

TABLE 2. Mean shoot dry-weight of Coker 237 soybean plant grown in soil amended with **Macrophomina phaseolina** and **Tridoderma harzianum** singularly, and in combination

Treatments	Shoot[a] dry weight (g)	Disease/Antagonism[b] %
Mp + Th	10.6	7.0
Mp Only	9.2	19.3
Th Only	13.2	+15.8
Control	11.4	--

[a]Each value represents the mean height of 7 experiments with 3 replicates each, and 8 plants/replicate.

[b]Each value represents the percent increase or decrease relative to control.

RESULTS AND DISCUSSION

Height and Dry-Weight of Coker 237 (untreated) Trials II

Effect of Pathogen/Antagonist on Shoot Height. Height of soybean plants grown in soil amended with **M phaseolina** and **T. harzianum** applied at planting was significantly reduced. The reduced growth indicates a pathogen/antagonist/host relationship (Table 1). Shoot height was reduced 6.8% relative to controls in pathogen/antagonist treatments, while a 15.1% and 7.3% reduction were noted for the pathogen only, and antagonist only treatments, respectively. Disease/antagonism incidence was expressed as percentage decrease or increase of growth relative to controls.

TABLE 1. Mean height of Coker 237 soybean plants grown in soil amended with **Macrophomina phaseolina** and **Trichoderma harzianum** singularly and in combination

Treatments	Shoot height[a] (cm)	Reduction/Increase[b] %
Mp + Th	52.0	6.8
Mp Only	47.4	15.1
Th Only	51.7	7.3
Control	55.8	--

[a]Each value represents the mean height of 7 experiments with 3 replicates, and 8 plant/replicates.

[b]Each value represents the percent decrease relative to controls.

Effect of Pathogen/Antagonist on Shoot-Dry Weight. Dry weight of shoots grown in soil amended with pathogen and antagonist applied at planting was significantly reduced relative to control (Table 2). Pathogen/antagonist treatments reduced dry-weight 6.4% relative to controls while the pathogen only, and the antagonist only reduced dry-weight 25.5% and 9.6% respectively. Treatment with the pathogen only, reduced dry-weight 20.5% relative to pathogen/antagonist treatments, and 3.4% with respect to the antagonist only.

TABLE 2. Mean shoot dry-weight of Coker 237 soybeans plants grown in soil amended with **Macrophomina phaseolina** and **Trichoderma harzianum** singularly, and in combination

Treatments	Shoot dry-weight[a] (g)	Reduction/Increase[b] %
Mp + Th	8.8	6.4
Mp Only	7.0	25.5
Th Only	8.5	9.6
Control	9.4	--

[a]Each value represents the mean height of 7 experiments with 3 replicates, and 8 plants/replicate.

[b]Each value represents the percent decrease relative to controls.

Effect of Pathogen/Antagonist on Root Dry-Weight. Dry-weight of roots grown in soil amended with Pathogen/Antagonist combinations applied at planting was significantly reduced relative to controls (Table 3). Dry weight was reduced 17.4% in pathogen/antagonist combinations, while a 30.4, and 21.7% reduction, respectively, was noted in pathogen only, and antagonist only treatments. Treatment with the pathogen only, reduced root dry weight 15.8% relative to pathogen/antagonist combinations, while the antagonist only treatment produced a 5.3% reduction over combinations.

TABLE 3. Mean root dry-weight of Coker 237 soybean plants grown in soil amended with **Macrophomina phaseolina** and **Trichoderma harzianum** singularly, and in combination

Treatments	Root dry-weight[a] (g)	Reduction/Increase[b] %
Mp + Th	1.9	17.4
Mp Only	1.6	30.4
Th Only	1.8	21.7
Control	2.3	- -

[a]Each value represents the mean height of 7 experiments with 3 replicates, and 8 plants/replicate.

[b]Each value represents the percent decrease of shoot dry-weight relative to controls.

RESULT AND DISCUSSION

Height and Dry-weight of Braxton (untreated) Trials I

Effect of Pathogen/Antagonist on Shoot Height. The Pathogen only, reduced shoot height 33.24% relative to the control (Table 1). In contrast, the antagonist and fungal combination reduced shoot height 40.19 and 27.79%, respectively. The expected pattern of plant/fungal behavior would be on the order of pathogen > fungal combinations > antagonist. The pattern was reversed when the antagonist reduced shoot height 23, and 36% more than the pathogen and fungal combination, respectively. The parasitic behavior of the antagonist is evident as in previous trials.

TABLE 1. Effect of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with the antagonist **Trichoderma harzianum**

Cultivar	Treatments	Shoot height[a] (cm)	Reduction[b] %
Braxton	Mp + Th	29.26	27.79
	Mp Only	27.05	33.24
	Th Only	23.02	43.19
	Control	40.52	- -

[a]Each value represents the mean shoot height of 8 experiments with 6 replicates, and 2 plants/replicate.

[b]Each value represents the percent decrease of shoot height relative to controls.

Effect of Pathogen/Antagonist on Shoot Dry-Weight. The Pathogen only, reduced shoot dry-weight 35.9% relative to the control, while the antagonist, and fungal combination reduced shoot dry-weight 64.43, and 47.51% respectively. Ostensibly, the antagonist behaved more as a parasite than the pathogen, and to a lesser degree than the fungal combination (Table 2).

TABLE 2. Effect of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with the antagonist **Trichoderma harzianum**

Cultivar	Treatments	Shoot Dry-weight[a] (g)	Reduction[b] %
Braxton	Mp + Th	0.95	47.51
	Mp Only	1.16	35.91
	Th Only	0.68	64.43
	Control	1.81	- -

[a]Each value represents the mean shoot dry-weight of 8 experiments with 6 replicates, and 2 plants/replicate.

[b]Each value represents the percent decrease of shoot dry-weight relative to controls.

Effect of Pathogen/Antagonist on Root Dry-Weight. The expected pattern of root dry-weight reduction in the order of pathogen > fungal combination > antagonist, did not develop (Table 3). The pattern of antagonist 56.76%]pathogen 40.5% developed. This indicates that the antagonist acted in some way that was detrimental to the soybean host, singularly, and in combination with the pathogen.

TABLE 3. Effect of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with antagonist **Trichoderma harzianum**

Cultivar	Treatments	Root Dry-Weight[a] (g)	Reduction[b] %
Braxton	Mp + Th	0.19	48.65
	Mp Only	0.22	40.54
	Th Only	0.16	56.76
	Control	0.37	- -

[a]Each value represents the mean root dry-weight of 8 experiments with 6 replicates, and 2 plants/replicate.

[b]Each value represents the percent decrease or increase of root dry-weight relative to controls.

RESULTS AND DISCUSSION

Height and Dry-weight of Braxton (untreated) Trials II

Effect of Pathogen/Antagonist on Shoot height. Shoot height was reduced 6.6% by the pathogen relative to the control, while the fungal combinations, and the antagonist reduced shoot height 11.8% and 15.6%, respectively (Table 1).

A 5.5% reduction in height occurred in fungal combination compared with the pathogen only, while a 9.6% reduction occurred when the pathogen only was compared with the antagonist only. Antagonist only trials reduced shoot height 4.3% relative to fungal combinations.

The expected pattern of shoot height reduction on the order of pathogen > fungal combinations > antagonist was not achieved. The order achieved was antagonist]fungal combinations]pathogen. this indicates that the data is inconclusive, or the antagonist is more parasitic than the pathogen.

TABLE 1. Effect of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with **Trichoderma harzianum**

Cultivar	Treatments	Shoot height[a] (cm)	Reduction[b] %
Braxton	Mp + Th	28.37	11.78
	Mp Only	30.03	6.62
	Th Only	27.15	15.58
	Control	32.16	- -

[a]Each value represents the mean shoot height of 6 combined experiments with 6 replicates, and 2 plants/replicate.

[b]Each value represents the percent decrease of shoot height relative to controls.

Effect of Pathogen/Antagonist on Shoot Dry-Weight. Shoot dry-weight was reduced 27.7% relative to the control, while fungal combinations, and the antagonist reduced shoot dry-weight 22.3 and 38.7%, respectively (Table 2).

A 6.1% reduction in shoot dry-weight occurred in fungal combinations relative to the pathogen only, while a 15.2% reduction occurred in antagonist only, relative to pathogen only trials. Antagonist only trials reduced shoot dry-weight 9.8% relative to fungal combinations.

The expected pattern of shoot dry-weight reduction; Pathogen > fungal combinations > antagonist was achieved with the former treatments. However, the antagonist reduced shoot dry-weight more than the other treatments relative to the control.

TABLE 2. Effect of charcoal rot caused by **Macrophomina phaseolina** on soybean amended with **Trichoderma harzianum**

Cultivar	Treatments	Shoot Dry-Weight[a] (g)	Reduction[b] %
Braxton	Mp + Th	0.93	22.34
	Mp Only	0.99	27.74
	Th Only	0.84	38.69
	Control	1.37	- -

[a]Each value represents the mean shoot dry-weight of 6 combined experiments with 6 replicates, and 2 plants/replicate.

[b]Each value represents the percent decrease of shoot dry-weight relative to controls.

Effect of Pathogen/Antagonist on Root Dry-Weight. Root dry-weight of the pathogen exceeded that of the control and the antagonist by 14.8%, and fungal combinations by 25.9% (Table 3). Root dry-weight was reduced 13% in fungal combinations relative to the control.

The expected order of reduction in root dry-weight was reversed by the pathogen in all treatment.

TABLE 3. Effect of charcoal rot caused by **Macrophomina phaseolina** on soybean in soil amended with **Trichoderma harzianum**

Cultivar	Treatments	Root Dry-Weight[a] (g)	Reduction/Increase[b] %
Braxton	Mp + Th	0.20	13.04
	Mp Only	0.27	+17.39
	Th Only	0.23	100.00
	Control	0.23	- -

[a]Each value represents the mean root dry-weight of 6 combined experiments with 6 replicates, and 2 plants/replicate.

[b]Each value represents the percent decrease or increase of root-dry-weight relative to controls.

EFFECTS OF HOPLOLAIMUS COLUMBUS ON MACROPHOMINA PHASEOLINA IN SOYBEAN

Effects of **Hoplolaimus columbus** *on* **Macrophomina phaseolina** in Soybean

Macrophomina phaseolina (Mp) is a facultative parasite of many plant species throughout the world (15). It is especially common on cultivated plant species and causes a seedling rot and also a mid-to late-season wilt of soybean (**Glycine max**) called charcoal rot (42). This disease occurs throughout the soybean growing areas of the world. The fungus is soilborne and survives as microsclerotia which may be naked in the soil or be present in plant debris. The fungus infects the roots of soybean plants with the rate of infection increasing with increased time after planting and with increasing soil temperatures (15,37). Infection frequency increases also when roots are wounded and the disease severity increases with increasing moisture stress on the plant (15).

Columbia lance nematode **(Hoplolaimus columbus)** (HC) occurs throughout the Coastal Plains soils of North Carolina, South Carolina, Georgia, and Alabama (33). Hc infects the roots of soybeans in both an ecto-and endoparasitic manner and migrates both on and within the roots as it feeds (32,33). Hc feeding severly wounds the root cortex and also damages the vascular tissue. This damage results in increased moisture stress, stunting, chlorosis, and premature senescence of soybeans.

Hc and Mp infest many of the same fields in the Coastal Plains soils of the Southeastern United States. Therefore, many plants are infected by both of these organisms since soybeans exhibit no resistance to either pathogen (20,31,38,42). A related nematode, **Hoplolaimus galeatus** appears to enhance the infection of cotton **(Gossypium spp)**by **Rhizoctonia solani** (7). Populations of **Rhizoctonia solani** in soybean roots during bloom is higher in plants infected with Hc than in noninfected plants (33).

Almost all of the changes that infection of soybeans by Hc induce in the host appear to make Hc infected plants more susceptible to infection by Mp and also more susceptible to the development of charcoal rot. Similarities between the environmental conditions which favor infection by both these organisms, and subsequent disease development exist which strongly indicate that a disease complex may exist, or that infection of soybeans by **H. columbus** may enhance the development of charcoal rot. Both organisms and the diseases they cause are enhanced by hot, dry weather. Wounding of soybean roots enhances infection of soybeans by Mp, and Hc causes extensive wounding of cortical tissues. Hc also induces moisture stress and premature senesence of the host, both of which enhance development of charcoal rot. Concommitant infection of a soybean by these two pathogens is very likely to occur, however, no studies have been performed to determine how infection of soybean roots by one of these pathogens will affect infection by the hand, and subsequent disease development.

MATERIALS AND METHODS

Greenhouse experiments were designed to determine if infection of soybeans by either Hc or Mp altered either infection by the other pathogen and/or development of the disease caused by either organism. A factorial set of treatments was established in the experiment involving the presence and absence of the two pathogens. The resulting four treatments were: Mp alone, Hc alone, Mp + Hc, and neither pathogen (nontreated check). The soybean cultivar used was "Braxton" which is highly susceptible to Hc and also susceptible to Mp. Inoculum of Hc was obtained from field-grown "Braxton" soybeans using a modified seinhorst mist apparatus. Inoculum of Mp was obtained by methods previously described.

Three "Braxton" soybean seeds were planted in 15 cm diameter plastic pots which contained autoclaved sandy-loam soil material. Beneath each seed was placed 1 ml of an aqueous suspension of Mp containing approximately 650,000 sclerotia.

After one week the stand in each pot was adjusted to two seedlings. At the same time 2500 juveniles of Hc were added at the base of each plant in slits which had been cut in the soil.

Six weeks after planting the plants were removed from the pots, the soil gently washed from the roots, and the level of infection by each pathogen measured. Mp infection levels were established by cutting 2-1cm pieces from the taproot of each plant, surface disinfecting each piece in 10% clorox for 4 minutes, then transferring one piece to Rose-Bengal agar and the other to Potato Dextrose-Agar. After one week the number of pieces from which a colony of Mp was growing was recorded. The infection level of Hc was established by placing the remaining roots in a modified seinhorst mist apparatus and after 7 days recording the number of Hc recovered per gram freshweight of root. Disease levels were evaluated by recording the fresh and dry weight of the epicotyls and roots. Data were analyzed using an analysis of variance, and a fisher's LSD test was used to compare treatment means.

Results

No Hc were recorded from the soil or roots of the check or Mp treatments. Recovery from the Hc treatment was soil 65 Hc per 250 cm 3 and 116 recovered per gram freshweight of root. In the Hc plus Mp there were 70 Hc per 250 cm 3 of soil and 117 Hc recovered per gram freshweight of root. Recovery of Mp from taproots was Mp 2%, check 4%, Hc 8%, and Mp + Hc 07%.

Stem freshweights were check, 3.28g, Mp 4.35g, Hc 3.94g, and Mp + Hc 4.11g. Stem dry weights check 2.29g, Mp 2.22g, Hc 2.32g, and Mp + Hc 2.41g. Differences between treatments within either the freshweights or dry weights were not statistically significant according to an FLSD test.

Root freshweights were check 2.98g, Mp 2.92g, Hc 2.85g, and Mp + Hc 2.88g. Root dry weights were check 0.27g, Mp 0.16g, Hc 0.28g, and Hc + Mp 0.21 g. Differences between treatments within either the freshweights or dry weights were not statistically significant according to an FLSD test.

Discussion

Some infection and reproduction of (LN) occurred in inoculated plants, however little infection by Mp appeared to occur, therefore, any affects of the two pathogens either alone or together were impossible to measure. Failure of Mp to infect may be due to the young age of the plants or the moisture conditions of the soil. Young soybean plants are often considered resistant to Mp and Mp normally has its greatest infection rate in relatively dry soils. Higher infection rates may be obtained if older plants are inoculated or if plants are allowed to age more prior to evaluation.

LITERATURE CITED

1. Adams, J. E., R. C. Wilson, L. E. Hessler, and D. R. Ergle. (1939). Chemistry and growth of cotton in relation to soil fertility and rootrot. Proc. Soil Sci. Soc. Amer. 4:329-332.

2. Albert, W. B. 1946. The Effects nutrient treatments have upon the resistance of cotton to to **Fusarium vasinfectum.** Phytopathology 36:703-716.

3. Allison, L. E. 1965. Organic Carbon. pages 1367-1378. In: Methods of soil analysis, part 2. Eds. C. A. Black D. D. Evans, J. L. White, L. E. Ensminger, and F. E. Clark. American Society of Agronomy, Inc., Madison, Wisconsin.

4. Anderson, P. J., A. V. Osmun, and W. L. Doran. 1926. Soil reaction and black root-rot of tobacco. Mass. Agr. Exp. Stn. Bull, 229:117-136.

5. Balis, C. and V. Kduyeas. 1968. Volatile inhibitors involved in soil mycostasis. Ann. Inst. Phytopathol. Benaki, H. S., 8:145-149.

6. Bristow, P. R., and Wyllie T. D. 1974. Enumeration of **Macrophomina phaseolina** propagules in field soils. Proc. Am. Phytopathol. Soc. 1:124

7. Brodie, B. B. and W. E. Cooper. 1964. Relation of parasitic nematodes to post-emergence damping-off of cotton. Phytopathology 54:1023-1027.

8. Butler, F. C. 1961. Root and foot rot disease of wheat. N. S. W. Depts. Agr. Sci. Bull. 77. 98 p.

9. Chang, Ya, Chang, Yih, Baker, R., Kleifeld, O., and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent **Trichoderma harzianum.** Plant Disease 70:145-148.

10. Critopoulos, P. D. 1953. A Contribution on the fungus flora of Greece. Torrey Bot. Club Bull. 80, pp. 325-341.

11. Cottingham, C. 1981. Ultrastructural formation of sclerotia of **Macrophomina phaseolina** in soybean. Pages 7-24 in : Biomass of **Macrophomina phaseolina** in South Carolina Soils and Soybean Host Tissue. Res. Bul. 19, South Carolina State College, Orangeburg, 32 pp.

12. Cottingham, C. 1981. Numbers and distribution of sclerotia of **Macrophomina phaseolina** in the soils of South Carolina. Plant Dis. 65:355-356.

13. Daines, R. H. 1937. Antagonistic action of **Trichoderma** on **Actinomyces scabies** and **Rhizoctonia solani.** Amer. Potato J. 14: 85-93.

14. Dennis, C. and J. Webster. 1971. Antagonistic properties of species groups of **Trichoderma** II. Production of volatile antibiotics. Trans. Br. Mycol. Soc. 57:41-48.

15. Dhingra, O. D. and J. B. Sinclair. 1978. Biology and Pathology of **Macrophomina phaseolina.** Universidade Federal de Vicosa, Vicosa, Brazil. 244 pp.

16. Doran, W. L. 1931. Increasing soil acidity as a means of controlling black root-rot of tobacco. Mass. Agr. Exp. Stn. Bull. 276:117-146.

17. Ebben, Marion H. 1971. Tomato brown rot: The build-up of soil inoculum and its control by fumigation. P. 243-250. Proc. 6th Br. Insecticide Fungicide Conf. (1971).

18. Elad, Y. Chet, I., and Katan, J. 1980. **Trichoderma harzianum'** A biocontrol agent effective against **Sclerotium rolfsii** and **Rhizoctonia solani.** phytopathology 70: 119-121.

19. Elad, Y., Hadgar, Y., Hadar, E., Chet, I., and Hennis, Y. 1981. Biological control of **Rhizoctonia solani** by **Trichoderma harzianum** in carnation. Plant Dis. 65:675-677.

20. Fassuliotis, G. 1974. Host range of the Columbia lance nematode **Hopoliamus columbus.** Plant Dis: Rep. 58:1000-1002.

21. Fenster, C. R., M. G. Boosalis, and J. L. Weihing. 1972. Date of planting studies of winter wheat and winter barley in relation to root and crown rot, gain yields and quality. Nebr. Agr. Exp. Stn. Res. Bull. 250, 32. p.

22. Ghaffar, A., G. A. Zentmyer, and D. C. Erwin. 1969. Effect of organic amendments on severity of **Macrophomina** root rot of cotton. Phytopathology 59:1267-1269.

23. Gilbert, W. W. 1909. The root-rot of tobacco caused by **Thielavi basicola.** U. S. Dept. Agr. Bur. Plant Ind. Bull. 158. 55 p.

14. Greaney, F. J. 1946. Influence of time, rate and depth of seeding on the incidence of root rot in wheat. Phytopathology 36:252-263.

15. Hardar, Y., Chet, I., and Henis, Y. 1979. Biological control of**Rhizoctonia solani** damping-off with wheat bran culture of **Trichoderma harzianum.** Phytopathology 69:64-68.

16. Hora T. S. and R. Baker. 1970. Volatile factor in soil fungistasis. Nature (London) 225:1071-1072.

17. Hora, T. S. and R. Baker. 1972. Soil fungistasis: Microflora producing a volatile inhibitor. Trans. Br. Mycol. Soc. 59:491-500.

18. Kilpatrick, R. A. 1955. Soybean diseases in the delta areas of Mississippi in 1954. Plant Dis. Reptr. 39:578-579.

19. Kilpatrick, R. A., and H. W. Johnson. 1953. Fungi isolated from soybean plants at Stoneville, Mississippi, in 1951-1952. Plant Dis. Reptr. 37:98-100.

20. Last, F. T., M. H. Ebben. 1966. The epidemiology of tomato brown root rot. Ann. App. Biol., 57:95-112.

21. Lewis, S. A., and F. H. Smith. 1976. Host plants, distribution, and ecological association of **Hoploliamus columbus.** J. Nematol. 8:264-270.

22. Lewis, S. A., F. H. Smith, and W. M. Powell. 1976. Host parasite relationship of **Hoplolaimus columbus** on cotton and soybeans. J. Nematol. 8:1141-1145.

23. Lewis, S. A. and G. Fassuliotis. 1982. "Lance Nematodes, **Hoplolaimus** ssp., in the Southern United States" pp. 127-138 in nematology in the southern region of the United States. Southern Cooperative Series Bulletin 276.

24. Meyer, W. A., Sinclair, J. B., and Khare, M. N. 1973. Biology of **Macrophomina phaseolina** in soil studied with selective media. Phytopathology 63:613-620.

Morse, W. J., Carter, J. L. 1937. Improvement in Soybeans. Pages 1154-1189 in: **U. S. Dept. Agr. Ybr. 1937.**

Morton, D. J. and W. H. Stroube. 1955. Antagonistic and stimulatory effects of micro-organisms upon **Sclerotium rolfsii.** Phytopathology 45:417-420.

37. Mueller, J. D., B. J. Shorer, and J. B. Sinclair. 1985. Effects of Cropping History, Cultivar, and Sampling Data on the Internal Fungi of Soybean Roots. Plant Disease 69:520-523.

38. Nyczepir, A. P., and S. A. Lewis. 1979. Relative tolerance of selected soybean cultivars to **Hoploliamus columbus** and possible effects of soil temperature. J. Nematol. 11:27-31.

39. Pettit, R. E., Ruth A. Tabler, and B. G. Foster. 1968. Occurrence of **Bacillus subtilis** in peanut kernels. Phytopathology 58:254-255.

40. Robinson, P. M. and D. Park. 1966. Volatile inhibitors of spore germination produced by fungi. Trans. Br. Mycol. Soc. 49:639-649.

41. Romine, Maureen, and R. Baker. 1973. Soil fungistasis: evidence for an inhibitory factor. Phytopathology 63:756-759.

42. Sinclair, J. B. ed. 1982. Compendium of Soybean Diseases. 2nd ed. American Phytopathological Society, St. Paul. MN. 104 pp.

43. Singh, O. V., V. K. Agarwal, and Y. L. Nene. 1973. Seed health studies in soybean raised in the Nainital Tarai. Indian Phytopath. 26:260-267.

44. Smith, A. M. 1973. Ethylene as a cause of soil fungistasis. Nature (London) 246:311-313.

45. Weindling, R. 1934. Studies on the lethal principle effective in the parasitic action of **Trichoderma lignorum** on **Rhizoctonia solani** and other fungi. Phytopath. 14:1153-1179.

46. Wells, H. D., D. K. Bell, and C. A. Jawarski. 1972. Efficacy of **Trichoderma harzianum** as a biocontrol for **Sclerotium rolfsii.** Phytopathology 62:442-447.

47. Willis, G. M. and L. E. Williams. 1968. Fungistatic properties of soils exposed to different antecedent environments. Can. J. Microbiol. 14:755-761.