To The Graduate Council:

I am submitting herewith a thesis written by Tamera D. Klingbyll entitled "In Vitro Inhibition of Phomopsis phaseoli by Microorganisms Acquired from the Environment of the Soybean." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of the Master of Science, with a major in Entomology and Plant Pathology.

James W. Hilty

James W. Hilty, Major Professor

We have read this thesis
and recommend its acceptance:

Mark T. Windham

R. N. Trigiano

Accepted for the Council:

Lou Winkel

Vice Provost
and Dean of The Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master of Science degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under the rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of the source is made.

Signature Tamera D. Klingfuß

Date December 12, 1989

IN VITRO INHIBITION OF *PHOMOPSIS PHASEOLI* BY MICROORGANISMS ACQUIRED FROM THE ENVIRONMENT OF THE SOYBEAN.

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Tamera D. Klingbyll

May 1990

DEDICATION

To my parents whose love and support made the impossible, possible.

ACKNOWLEDGEMENTS

In the course of thesis research, a number of scholars have assisted me generously. In particular I am indebted to Professors James Hilty, Mark Windham, and Bob Trigiano, who served as my director and committee respectively. I was able to achieve results otherwise impossible. Whatever errors remain in my work, however, are solely my responsibility.

I would also like to thank these other people who have given me help:

Dr. William Sanders and John Schneider in their assistance in the evaluation of my statistical work,

Dr. Larry W. Barnes of Texas A & M for running fatty acid profiles of my bacterial isolates,

Dr. Kim Gwinn for her assistance in the C/N study,

Mary Montgomery for the support that only a friend could give,

Dee Ann Ostby, Becky Collins, and Mary Collins-Shepard for sharing space and time with a graduate student,

And to Jesus Christ my LORD for without whom I would not be.

ABSTRACT

Stem canker of soybean is caused by Diaporthe phaseolorum var. caulivora and its conidial stage, Phomopsis phaseoli. Stem canker is currently controlled by fungicides, crop rotation, resistant soybean cultivars, and tillage practices. Biocontrol agents such as Bacillus cereus, Gliocladium roseum, and Trichoderma species have been used as successful controls of plant diseases related to Diaporthe and Phomopsis.

Microorganisms isolated from the phylloplane, rhizosphere, and seed pods were reinoculated onto soybean leaves, stems, and seed; those not pathogenic to soybean were tested against P. phaseoli mycelium and spores in vitro. Cross culture methods were used to examine the effects of temperature, C/N ratios, and hyperparasitism. Zones of inhibition were measured to determine if antibiosis occurred in the temperature and C/N studies. Culture filtrates of the isolates were used in spore germination tests.

Twelve microorganisms were used in experimentation with one of the organisms having been collected a previous year. Isolates produced their largest zones of inhibition at 22 and 24 C. The largest zones of inhibition produced by the isolates during the carbon/nitrogen study developed when carbon was absent or deficient or when nitrogen was

absent. Significantly fewer P. phaseoli spores germinated at a concentration of 5,000 spores per ml, when placed in isolate filtrates. Hyperparasitism did not occur between any isolate and P. phaseoli. Three Bacillus pumilus isolates produced results as potential biocontrol agents. Isolates D.1 and D.T collected from the phylloplane produced significantly larger zones of inhibition from the P. phaseoli control in the temperature and C/N studies. Isolate D.T also significantly reduced the number of P. phaseoli spores that germinated in the germination study. Isolate FSII, Bacillus pumilus, from the rhizosphere, produced significantly larger zones of inhibition in the temperature and nutrition studies, however, did not significantly reduce the germination of P. phaseoli spores in the germination study. These isolates need to be further examined to determine their potential as future biocontrol agents and as a part of integrated systems.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Stem canker of soybean, Glycine max (L.) Merr., caused by Diaporthe phaseolorum (Cke. and Ell.) Sacc. var. caulivora Athow and Caldwell and the conidial stage, Phomopsis phaseoli (Desm.) Sacc., was first found in the midwestern United States in the 1940's (75). The pathogen has since spread throughout the north central and southeastern states (55). Estimated losses due to stem canker in the south during 1987 were 3.4 million bushels of soybean or 18.7 million dollars (55). Stem canker isolates have been inhibited by the use of resistant soybean cultivars (2,3,9,38,62), fungicides (2,25,49,50), crop rotation, and tillage practices (3,61,64).

Symptoms Caused

The initial symptoms of stem canker on seedlings are small reddish-brown lesions which develop on one or both cotyledons. Symptom expression increases as discoloration develops in the stems. Seedlings may wither and die due to stem girdling (69).

Maturing infected plants can be recognized by reddish-brown lesions on leaf scars. Lesions enlarge rapidly and, in many cases, girdle the stem causing the plant to die. Lesions turn brown or black with age. Stem canker can be

distinguished in the field from other soybean diseases because dead or dying plants retain dead leaves (1,69).

Yield reduction is dependant on when the plant becomes infected. An early infection can cause plant death prior to flowering, and those which are infected during pod fill, may produce smaller seed (21).

Disease Cycle

Disease cycles of D. phaseolorum var. caulivora and P. phaseoli overlap. Inoculum is produced on overwintering soybean debris (32,41,49,69,75) and alternate hosts (21,24,63). The source of inoculum has little effect on the development of disease (57). Inoculum is disseminated by splashing (3,41) and air currents (69). Primary infection courts for D. phaseolorum var. caulivora are petiole bases and stems (14,15,49,60). Kulick (44) discovered that germ tubes of P. phaseoli conidia formed appressoria and penetrated leaves via stomata. The pathogen within the young plant does not move up the plant as the plant matures (21,32,41). Kmetz et al. (41) considered that pod infection occurred independently of stem infection. Reproductive structures are formed, once established within the field (22).

Inoculum can also be produced within seed (36,56,68,69). The fungus grows within the I cell layer of the seed coat and remains dormant until conditions are

favorable for seed germination (36,69). The fungus can inhibit seed germination, reduce plant vigor, and predispose the seedling to other pathogens (36).

Many factors which have an effect on disease development are temperature (6,37,39,58,59,71), moisture (6,64,71), plant age (28,42,73), insect wounds (65,66,67), and toxicity of the isolate of the pathogen (7,8). Pathogenicity of D. phaseolorum var. caulivora on soybean seedlings occurs between 21 and 30 C (39). Seed infection increases in hot, humid weather (6). During these humid periods, water is increased within the seed and can cause the seed coat to crack, predisposing the seed to invasion (64). As the temperature declines to 21-15 C longer periods of high humidity are required for the invasion of the seed coat. Disease severity and seed invasion therefore increase the longer seed is left in the field (28,42). Insects which feed on seed predispose the seed to invasion (67); however, defoliation by some insects reduces the severity of stem canker (66). Different isolates of D. phaseolorum var. caulivora produce different amounts of toxin which causes variation in the severity of the canker produced (8).

Life Cycle

Phomopsis and Diaporthe differ in their reproductive phases, yet, are similar in their somatic phases. Phomopsis

produces alpha and beta spores within pycnidia (3,69). Diaporthe phaseolorum var. caulivora forms black, globose perithecia in caespitose groups of 2-12 stroma. Asci are sessile, elongate clavate and have thin evanescent walls which are slightly thickened at the base (54,69). Ascospores are hyaline, elongate ellipsoid, two-celled, slightly constricted at the septum, and biguttulate (69). Diaporthe is homothallic and perithecia can develop from single ascospore cultures (69,75).

Ascospores and conidia swell in a humid environment. A germ tube is produced and the guttulae disappear. The germ tube increases in length and penetrates the host tissue. Mycelia invade the cortex, inter- and intracellularly (46,74). Evidence of stelar tissue invasion is seen as a darkening of the trachea and pith.

History of the Disease

Prior to 1948, pod and stem blight and stem canker were thought to be caused by the same fungus (3). In 1920 Wolf and Lehman (76) discussed a Phoma blight which occurred on soybean pods and stems. Lehman (45) later discovered the perithecial stage of the Phoma and named it Diaporthe sojae Lehman. Wehmeyer (74) in 1933 did a taxonomic study of D. sojae, and changed the name to Diaporthe phaseolorum var. sojae. In 1947 Luttrell (48) compared the morphology, culture growth, pathogenicity, and host range of

D. phaseolorum and D. phaseolorum var. sojae. Luttrell concluded that both were common on stems and petioles; however, the two were different on the basis of culture growth and pathogenicity.

Welch and Gilman (75) discovered two morphologically and pathogenically distinct types of Diaporthe perithecia on overwintering soybean stems. The two were named D. phaseolorum var. sojae, pod and stem blight fungus, and for the stem canker fungus a strain of D. phaseolorum var. batatatis, which was first discovered on sweet potato. In 1949 Crall (13) observed that D. phaseolorum var. batatatis was one of two important diseases on soybean in Iowa and suggested that "stem canker" be the descriptive name of the disease. Athow and Caldwell (1) confirmed Welch and Gilman's findings, yet, changed the name of the stem canker fungus to D. phaseolorum var. caulivora because of perithecia and asci size. Hildebrand (31) discovered a conidial stage of alpha spores for D. phaseolorum var. caulivora. Beta spores were described by Frosheiser (21). The presence of conidia was concluded not to be important in the incitement of disease (32). Kmetz, et al. (40) found that both the Diaporthe stage and the Phomopsis stage were virulent on soybean seed. However, Phomopsis was more virulent on seed; whereas, Diaporthe produced girdling stem cankers on susceptible soybean plants.

The first report of D. phaseolorum var. caulivora in the south was in Mississippi during 1973 (3). Stem canker was later discovered in Alabama (24), Tennessee (33), South Carolina (43), and Arkansas (35). Morgan-Jones and Backman (53) became the first to observe morphological and physiological differences between the midwestern/north central and southeastern biotypes of D. phaseolorum var. caulivora. Backman et al. (3) suggested separating the two biotypes by making them forma specialis. Other confirmations of differences between the two races have been made (29,30,39,41). Keeling in 1984 and 1985 (37) found six pathogenic races of the southeastern type, and Hilty (34) found differences in pathogenicity in several isolates of D. phaseolorum var. caulivora in Tennessee.

Biocontrol of Plant Pathogens

Cook and Baker (11) defined biocontrol as "... the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man." Biocontrol agents primarily work within two pathways. The first involves the biocontrol agent and its interactions with the host which are expressed as crop protection and hypovirulence (4). The second involves the biocontrol agent and its interactions with the pathogen (antibiosis, competition, and exploitation) (4). In most studies antagonists are found in the area where

the control is to take place (70). Factors, environmental and human intervention, which effect the pathogen also effect the biocontrol agent (5,26).

Many biocontrol agents have been noted by Cook and Baker (11), and many of the reported agents are for the control of soil-borne pathogens. Soil-borne pathogens which are related to P. phaseoli have been controlled by the use of antagonists. In the 1970's Phomopsis sclerotioides Van Kesteren, causing cucumber black root rot, became persistant in European greenhouses even with the use of known chemicals and steam control. Gliocladium roseum Bainier and Trichoderma spp. were found to be antagonists of P. sclerotioides (51,72). Two other potential antagonists found were Penicillium lilacinum Thom. and Streptomyces griesus (Krainsky) Waksman and Henrici (18).

Another Phomopsis-related soil-borne pathogen, Gaeumannomyces gramminis var. tritici has been inhibited by Bacillus cereus Frankland and Frankland, and potentially inhibited by Pythium oligandrium Dres. and Streptomyces lavendulae (Waks. and Curt.) Waksman and Henrici (11).

Studies have also been made with the control of aerial pathogens. Upper aerial plant surfaces are covered with rain- and air-borne propagules; whereas, lower plant parts are splashed with soil particles and microorganisms. Aerial microorganisms usually compete for nutrients and

space (12). Wood (77) discovered competition occurred between Botrytis cinerea Pers. and several microorganisms when placed on lettuce. Fravel and Spurr (20) also studied the potential control of an aerial pathogen. They examined the biocontrol on the germination and growth of Alternaria conidia germ tubes by selected bacteria. In vitro screening, the bacterial isolates of Pseudomonas maltophilia Hugh and Rych. and Bacillus cereus subsp. mycoides were found to reduce the number and length of germ tubes, the branching on the longest germ tube, and the number of appressoria produced. The filtrates of these bacteria produced no differences.

Chaetomium cupreum Ames, in cross culture and filtrate culture, inhibited mycelial growth of Fusarium sp., Macrophomina phaseolina, Phomopsis sp., and Rhizoctonia solani, pathogens which occur on soybean seed (17,78). When the filtrate of C. cupreum was purified and concentrated, Alternaria sp., Cercospora sojina, and Gliocladium roseum mycelium were also inhibited. However, the fungitoxic compound produced by C. cupreum also reduced soybean seed germination (78).

Cubeta et al. (16) found that Bacillus subtilis or its culture filtrate, when applied as a seed treatment, inhibited Phomopsis sp. in vitro, but not in vivo. Similar to C. cupreum, B. subtilis and its filtrate reduced soybean

seed emergence, and B. subtilis reduced plant vigor.

Biocontrol agents from the soybean could compete in their environment, and not only compete with Phomopsis and Diaporthe, but also other soybean pathogens. Chemical treatments are being taken off the market due to their detrimental effects on the environment, biocontrol agents (fungi, bacteria, nematodes, etc.) are a part of the environment and would go back into the ecosystem. The objective of this study was to isolate microorganisms that demonstrate the ability to inhibit the growth of P. phaseoli and to investigate their potential as biocontrol agents.

CHAPTER II

MATERIALS AND METHODS

Microbe Collection

Microorganisms were isolated from the phylloplane, rhizosphere, and seed pods of various cultivars of soybeans from test fields on the University of Tennessee Plant Science Farm.

Phylloplane. A method for isolation described by Sleesman and Leben (70) was modified and used. Twenty-one 1 cm discs were cut from leaves using a sterile cork borer and ground with 7.0 ml of sterile deionized water in a sterile mortar. The liquid from the homogenate was then diluted to 1/1000 with sterile deionized water. One tenth ml of the 1/1000 dilution was spread over the surface of each of five Petri plates containing Difco potato dextrose agar (PDA) with the tip of a pipet, and incubated at 24±1 C for seven days. A Phomopsis phaseoli conidial suspension was atomized over plates containing individual colonies. Conidia were obtained from mycelium on soybean stems in Petri plates. After 14 days at 24±1 C, organisms exhibiting zones of inhibition were isolated on PDA and maintained in culture bottles containing PDA at 24±1 C.

Rhizosphere. Soil immediately surrounding soybean roots was diluted with sterile deionized water at dilutions

of 1/10, 1/100, and 1/1000. One tenth ml of each dilution was spread on five PDA plates incubated at 24±1 C for seven days. Individual colonies were oversprayed, and colonies producing inhibition zones were maintained as described.

Seed pods. Six green pods randomly collected from field-grown soybeans were washed in running tap water with four drops of Tween 20 for ten minutes and then rinsed in sterile deionized water. All pods and seeds were crushed in 10 ml of sterile deionized water in a sterile mortar. The liquid portion of the homogenate was diluted to 1/10, 1/100, 1/1000, and 1/10,000 with sterile deionized water, and one tenth ml of each dilution was spread with the tip of a pipet on three PDA plates. The plates were incubated at 24±1 C for seven days. Individual colonies were oversprayed with P. phaseoli spores and colonies producing zones of inhibition were maintained as described.

Pathogenicity Tests of Isolates to Soybean

Each microorganism with inhibitory qualities was tested for pathogenicity on the Essex cultivar of soybean in greenhouse studies, which were initiated in late May. Crall's (14) toothpick method of stem inoculation was modified to determine if any of the isolates could infect stem regions. Flat toothpicks were boiled in three changes of sterile, deionized water and placed in Petri plates containing 10 ml of Difco potato dextrose broth (PDB). The

broth and toothpicks were then sterilized and plugs of agar containing either the mycelium or bacterial cells of the isolates were placed in Petri plates of broth. Toothpicks covered with P. phaseoli mycelium, known to produce cankers, and sterile broth were used as controls. Isolates were added to separate plates and incubated at 24±1 C. When colonies covered the toothpicks, about 14 days, toothpicks were inserted in punctures made in the hypocotyl 1-2 cm below the cotyledon node. Punctures were made with a sterile dissecting needle. Twenty, 21 day-old soybeans were inoculated with each isolate and controls. After 50 days of greenhouse incubation, one stem from each pot was cut lengthwise and evaluated for internal lesion development.

Each isolate was tested as a foliar pathogen. Ten, 10 day-old soybean plants, one per pot, were atomized to run-off with either a conidial suspension from fungal isolates or a mixture of bacterial isolate spores and cells in sterile deionized water at concentrations of approximately four million spores per ml. P. phaseoli conidia at a concentration of four million spores per ml and sterile broth were atomized as controls. Inoculated plants were placed in the greenhouse and covered overnight with plastic bags. Lack of symptom development after 36 days indicated no pathogenicity.

Isolates were examined as possible seed pathogens.

A modified method of seed inoculation described by Hadar et al. (27) was used. Conidia and bacterial spores were scraped from 9-14 day old colonies on PDA, diluted to concentrations of 10^8 spores per ml, and placed in flasks containing 500 ml of PDB. P. phaseoli conidia and sterile broth were treated similarly and used as controls. Two hundred seeds for each isolate and each control were suspended five minutes in the respective spore suspensions. Seeds were air-dried for 14 hours and then planted 1/2 in. deep in flats containing sterile 2 PROMIX : 1 sand. Percent emergence was recorded in 10 days.

Isolate Evaluation

Those isolates not pathogenic to soybean were then tested against P. phaseoli in laboratory tests. Cross cultures (17), germination (20), and hyperparasitism (47) trials were performed to determine how each isolate affected P. phaseoli mycelium and conidia. P. phaseoli was used because it can incite disease on stems and seed.

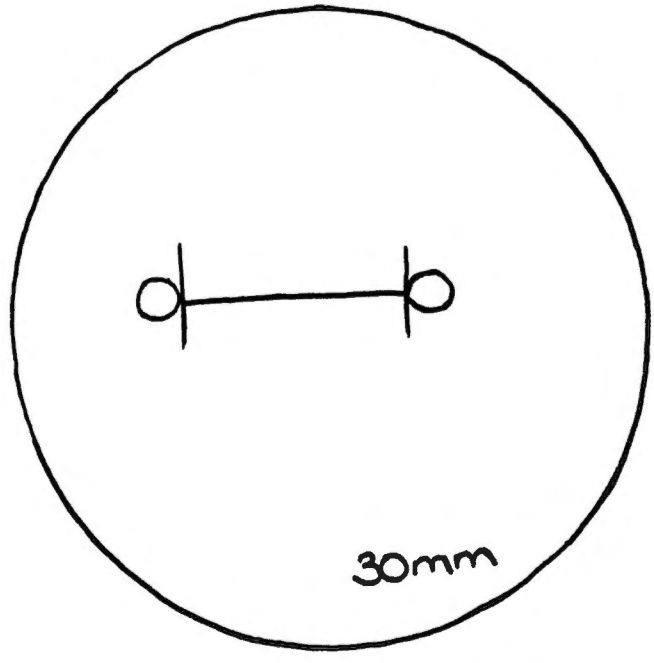
Cross cultures. A cross culture method for examining growth patterns of two organisms as the two meet was described by Dhingra and Sinclair (17). This study examined the effect of temperature, and the effects of carbon/nitrogen (C/N) ratios in a defined medium. P. phaseoli was placed opposite P. phaseoli and used as a control in both experiments. The initial measurements were made from the

inner edge of each opponent plug, and all following measurements were taken in the same plane as the first, but at the edge of each opponent colony's growth (Figure I).

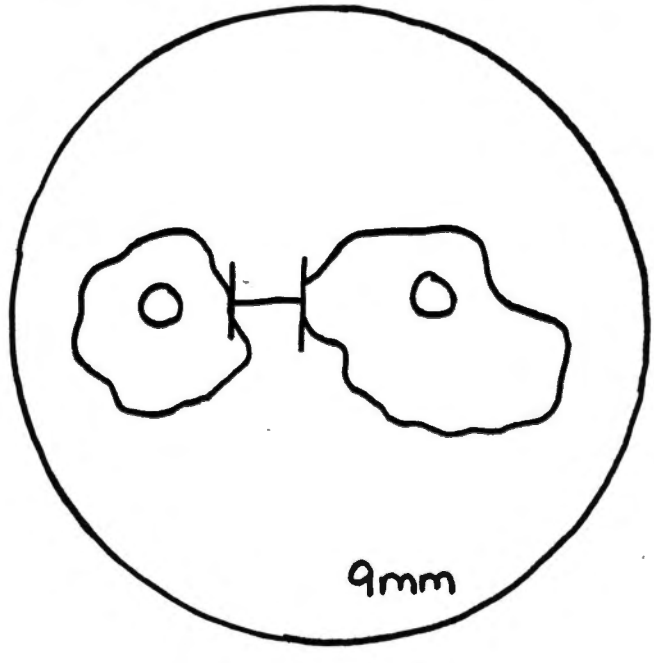
1. Temperature effects. Treatments for this study were 22, 24, 26, 28, 30, and 32 C. These temperatures were chosen to include optimum temperatures of 28-34 C for disease development caused by P. phaseoli (59). The experimental design was a computer generated randomized incomplete block with four replications. Each of six blocks consisted of four temperatures and was maintained for 14 days. Each isolate-versus-P. phaseoli test consisted of five plates.

Seven millimeter (mm) plugs were cut with a sterile cork borer from the perimeter of seven day-old isolate cultures and placed mycelium side down on one side of 9 cm PDA Petri plates. A 7 mm plug of P. phaseoli was placed approximately 30 mm opposite from the isolate. Plates were placed in plastic bags in appropriate incubators. Zones of inhibition were measured after 2, 7, and 14 days, with the measurements of zones after 14 days used for analyses. The measurements after two days were to assure organism growth; whereas, the measurements after seven days were to determine if a decrease in the zone had occurred.

2. Nutrition effects. Modified Fries (17) medium was used. Five replications were made of each C/N ratio



(A.) Initial measurement taken in cross culture studies.



(B.) Method for taking all remaining measurements in cross culture studies.

Figure I. Cross culture measuring technique.

(Table I) in 9 cm Petri plates for each test. A 7 mm plug of an isolate was placed opposite from a seven mm plug of P. phaseoli. P. phaseoli was used as a control when placed opposite itself. The plates were placed in plastic bags, incubated for 14 days at 24±1 C, and after 14 days zones were measured.

Germination of conidia. A method for measuring extracellular inhibition of conidia germination described by Fravel and Spurr (20) was used. Isolates were incubated 7 days in shake cultures in 250 ml flasks containing 150 ml of PDB. Cultures were filtered, and 0.05 ml of each filtrate was mixed with an equal volume of each of six concentrations of P. phaseoli conidia and placed in depression slides. The conidial concentrations used were 1000, 5000, 10,000, 50,000, 100,000, and 500,000 spores per ml. Five depression wells were used for each isolate. The experimental design was a randomized incomplete block, and each treatment was replicated three times. Treatments were divided eight per each of six blocks for each P. phaseoli concentration. P. phaseoli was the control for this study and was treated in the same manner as the isolates.

Liquid placed on slides was allowed to dry for two hours under an active laminar flow hood. Slides were then placed at 100 percent relative humidity in an incubator at 24±1 C. Hilty (unpublished) discovered that more P.

Table I. Grams of carbon to nitrogen in each liter of modified fries medium used in C/N effects study.

Medium	Sucrose (grams)	:	Sodium Nitrate (grams)
1	30.00		0.00
2	30.00		0.01
3	30.00		2.00
4	0.10		2.00
5	0.00		2.00

phaseoli conidia germinated at 24 C than at 16, 20, 28, and 32 C. After 60-70 hours, three drops of deionized water were placed in each depression and covered with a coverslip. Spore germination was observed using a light microscope 40 X objective. Ten spores per depression were examined for the presence of germ tubes.

Hyperparasitism. A form of cross culture testing was modified for hyperparasitism studies. This modified form had been used by Lifshitz et al. (47). The controls used were Trichoderma, a known hyperparasite, Rhizoctonia, a host to Trichderma, and P. phaseoli. Bacteria were excluded from testing. Using a permanent marker, the bottom of a 9 cm Petri plate was traced onto a sheet of cellophane (Decco film). The inside of each circle (approximately 8.5 cm diameter disc) was cut and boiled in deionized water for an hour, rinsed in three changes of deionized water, and autoclaved.

A sterile piece of cellophane was smoothed over the surface of one percent PDA in a 9 cm Petri plate, using a bent "7" shaped glass rod. A seven mm diameter plug, cut from a random location in the culture of a seven day old isolate plate was placed opposite from a seven mm P. phaseoli plug. Plates were placed in plastic bags, sealed, and incubated at 24±1 C. After the colonies merged, an approximately 2x4 cm rectangle of cellophane at the point of

merger of cultures was cut and examined under a light microscope for hyperparasitism. Each of three plates was counted as a replication for each test.

Data Analysis

All numerical figures, including zeros, taken as data were analyzed. Zero measurements represent three situations. The first situation occurs when the isolate and P. phaseoli had grown together in the test plate. The second situation occurs when the isolate has overgrown P. phaseoli before P. phaseoli has begun to grow. The final situation occurs when the isolate and P. phaseoli are growing within the plate, however, the isolate produces spores which disseminate and begin new colonies within the zone between the isolate and P. phaseoli.

Temperature Effects. Measurements of the zones of inhibition were analyzed using Duncan's Multiple Range Test (P=0.05) using the natural log of the zone + 1. The first analysis made to temperature data was on the effects of each temperature on all tests organisms. The second analysis involved the effects of each isolate when all temperatures were considered. Those isolates which wre significantly larger than the P. phaseoli control were then analyzed separately to determine at which temperature the larger zones of inhibition occurred. All tabular data was converted back to millimeter measurments for consistant reading.

C/N Effects. Measurements of the zones of inhibition were analyzed using Duncan's Multiple Range Test (P=0.05) using the zone. When all isolates were considered, an analysis was made on the effects of each C/N medium. The second analysis was made on the effects of each isolate at 24 C, considering all C/N media. The final analysis was made on those isolates which were significantly larger than the P. phaseoli control. These isolates were analyzed separately to determine at which medium the larger zones of inhibition occurred.

Germination. The concentrations of spores at which significant differences occurred between the isolates and the P. phaseoli controls were reported. All data was analyzed according to Duncan's Multiple Range Test (P=0.05) using the average number of spores germinated for each replication.

CHAPTER III

RESULTS

Microbe Collection

Eleven microorganisms that inhibited growth of *P. phaseoli* in culture were acquired from the environment of the soybean plant. A *P. phaseoli* culture, obtained from the Essex variety of soybean, was used as a control in all experiments, and a previously acquired bacterium from the Forrest variety of soybean seed was added to the eleven microorganisms as a possible inhibitor of *P. phaseoli* (Table II).

Pathogenicity of Isolates to Soybean

Only isolate 10, *Phomopsis* sp., was eliminated after performing greenhouse tests. Isolate 10 produced stem canker-like symptoms when inoculated into soybean stems. The *P. phaseoli* control plants had canker lengths ranging 5.7-42.0 cm compared to the canker lengths produced by isolate 10 of 18.0-46.4 cm.

The *P. phaseoli* control did not produce any foliar symptoms on the leaves or petiole bases. Some areas of discoloration were seen in the soybean leaves for many of the isolates; however, these were similar to other discolorations found on the water control leaf surfaces and were proposed to have been caused by the atomization

Table II. Microorganisms isolated from the phylloplane, rhizosphere, and seed pods of soybean.

Origin	Code	Organism
Phylloplane	2	Monocillium sp.
	3	Monocillium sp.
	5	Penicillium sp.
	6	Monocillium sp.
	D.1	Bacillus pumilus
	D.T	B. pumilus
Rhizosphere	A	Epicoccum sp.
	B	Epicoccum sp.
	C	Penicillium sp.
Seed Pods	7	Fusarium oxysporum
	10	Phomopsis sp.
	FSII[z]	B. pumilus

[z] A previously acquired bacterium.

technique. None of the isolates or controls inhibited seed germination or induced damping-off.

Analyzed Zeros

Many tested isolates produced 0.0 mm measurements. The Monocillium spp., isolates 2, 3, and 6, and the Penicillium spp., isolates 5 and C, were rapid spore producers, whose spores disseminated over the surface of the plates. Colony margins for Fusarium oxysporum and the margins of P. phaseoli met after 14 days. This phenomena was also seen to occur for some test plates of isolates A and B. All isolates of Bacillus pumilus had plates in which the bacterium overgrew the P. phaseoli colony.

Temperature Effects on Zones of Inhibition

Considering all isolates, the largest zones of inhibition between antagonists and P. phaseoli colonies occurred at 22 and 24 C with width means of 0.5 mm. The measurements at 22 and 24 C were only significantly larger from the width mean at 26C.

Considering all temperatures, zones of inhibition of six antagonists were significantly larger from the zone mean of the P. phaseoli control of 0.0 mm, when zones of inhibition of each antagonist were analyzed (Table III). The temperature at which the largest zones of inhibition were produced for each of the six antagonists are

Table III. Effect of temperature on zones of inhibition between antagonistic organisms and *P. phaseoli* on potato dextrose agar.

Organism	Code	Colony Distance
Monocillium sp.	2	0.1[Y] def[Z]
	3	0.0 ef
	6	0.2 def
Penicillium sp.	5	0.4 de
	C	0.1 def
Fusarium oxysporum	7	0.1 def
Epicoccum sp.	A	1.5 a
	B	1.1 ab
Bacillus pumilus	D.1	1.2 ab
	D.T	0.9 bc
	FSII	1.4 ab
Phomopsis phaseoli	P	0.0 f

[Y] Means are from four replications, 30 plates per replication. Temperature specification was omitted.

[Z] Numbers followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05). The log of the zones were used in data analysis. Zone means presented are average widths at all temperatures. Data has been converted back to milliliters.

presented in Table IV. The millimeter measurments are converted from the natural log of the zone + 1, so that measurements may be reported consistantly throughout the text. Isolate 5 produced largest zones at 22 and 32 C with zone means of 1.1 mm and 0.6 mm, respectively. Significantly larger zones of inhibition were produced by Epicoccum isolate A at 24 and 28 C with zone means of 1.9 and 3.3 mm, respectively. Epicoccum isolate B produced significantly larger zones of inhibition at 24, 28, and 30 C with zone means of 1.6, 1.6, and 1.3 mm, respectively. Isolate D.1, identified as Bacillus pumilus by a fatty acid analysis, produced its largest zone of inhibition, 1.8 mm, at 24 C, but was only significantly larger than zones developed at 26 C. Isolate D.T produced its largest zones of inhibition at 32 C with a zone mean of 1.5 mm; however, this mean was only significantly larger 26 and 28 C with zone means of 0.1 and 0.2 mm, respectively. The Bacillus pumilus isolate, coded FSII, produced its largest zone of inhibition mean, 1.8 mm, at 28 C, which was only significantly larger than 26 C.

C/N Effects on Zones of Inhibition

Zones of inhibition of all organisms were significantly larger when carbon or nitrogen was absent or when carbon was deficient from the medium (Table V) when compared to the zones of inhibition developed on Modified

Table IV. Effect of temperature on width of zones of inhibition between selected antagonistic organisms[x] and P. phaseoli on potato dextrose agar.

Temperature	Penicillum sp.	Epicoccum sp.		Bacillus pumilus		
(C)	5	A	B	D.1	D.T	FSII
22	1.1[y]a[z]	1.7 b	0.6 bc	1.1 a	0.8 ab	0.6 ab
24	0.4 bc	1.9 ab	1.6 a	1.8 a	0.7 ab	1.6 ab
26	0.0 c	0.5 cd	0.4 c	0.3 b	0.1 c	0.6 b
28	0.0 c	3.3 a	1.6 a	0.8 ab	0.2 bc	1.8 a
30	0.1 c	1.2 bc	1.3 ab	0.8 ab	0.9 ab	1.5 ab
32	0.6 ab	0.3 d	0.2 c	1.5 a	1.5 a	1.5 ab

[x] Fungi that produced zones of inhibition significantly larger than the control.

[y] Means are from four replications, five plates per replication. All means are in millimeter measurments.

[z] Numbers in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05).

Table V. Effects of C/N ratios on zones of inhibition when all isolates were tested.

C/N Amounts[x]	Zone Mean (mm)
30.00/0.00	0.5[y] ab[z]
30.00/0.01	0.3 bc
30.00/2.00	0.2 c
0.10/2.00	0.6 a
0.00/2.00	0.7 a

[x] Gram amounts for each of five media used.

[y] Means are from three replications, 80 plates per replication.

[z] Numbers followed by the same letter are not significantly different (P=0.05) according to Duncan's Multiple Range Test.

Fries medium containing the recommended ratios (17).

The zone of inhibition means of each isolate versus P. phaseoli test, considering all C/N media, are presented in Table VI. Zones of inhibition of isolates 5, D.1, D.T, and FSII were significantly larger than the zones produced by the P. phaseoli control. The analysis of each of these isolates is presented in Table VII.

Isolate 5 was the only isolate to produce significantly larger zones than P. phaseoli with the largest zones of inhibition being produced at the recommended C/N ratios for Modified Fries medium. The zones of inhibition of D.1 were significantly higher when carbon was absent, a 1.3 mm average, or deficient, a 1.9 mm average. Isolate D.T produced similar results to isolate D.1 with the largest significant zones occurring when carbon was absent or deficient (Table VII). Isolate FSII produced significantly larger zones of inhibition, a 7.4 mm average, when nitrogen was absent.

Effects of Filtrates on Spore Germination

Significant differences between antagonists and the P. phaseoli control were only found at the spore concentration of 5,000 per ml (Table VIII). The average amount of spores which germinated in the P. phaseoli control was nearly 20 percent, and this level of germination was consistant in both trials of P. phaseoli filtrate controls. Percent

Table VI. Width of zones of inhibition between antagonistic organisms and P. phaseoli as effected by C/N ratios at 24 C.

Organism	Code	Zone Mean (mm)
Monocillium sp.	2	0.0[Y] d[Z]
	3	0.0 d
	6	0.0 d
Penicillium sp.	5	0.3 cd
	C	0.0 d
Fusarium oxysporum	7	0.0 d
Epicoccum sp.	A	0.0 d
	B	0.0 d
Bacillus pumilus	D.1	0.7 c
	D.T	1.1 b
	FSII	4.2 a
Phomopsis phaseoli	P	0.0 d

[Y] Means are from three replications, 25 plates per replication. Zone width means are averages at all C/N ratios tested.

[Z] Numbers followed by the same letter are not significantly different (P=0.05) according to Duncan's Multiple Range Test.

Table VII. Zone widths between antagonists and P. phaseoli at different C/N ratios at 24 C for 14 days.

	Penicillium sp.	Bacillus pumilus		
C/N[x]	5	D.1	D.T	FSII
30.00/0.00	0.0[y] b[z]	0.0 b	0.0 b	7.4 a
30.00/0.01	0.0 b	0.0 b	0.4 b	4.0 bc
30.00/2.00	1.0 a	0.1 b	0.1 b	1.9 c
0.10/2.00	0.4 b	1.9 a	2.5 a	3.2 bc
0.00/2.00	0.0 b	1.3 a	2.7 a	4.6 b

[x] C/N amounts in grams used in each medium.

[y] Means are from two repetitions, five plates per repetition. Means are in millimeter measurments.

[z] Numbers followed by the same number are not significantly different (P=0.05) according to Duncan's Multiple Range Test.

Table VIII. Effects of culture filtrates of antagonistic organisms on germination of *P. phaseoli* spores.[x]

Organism	Code	Germination Mean
Monocillium sp.	2	1.4[y] bcd[z]
	3	1.3 cd
	6	1.7 abc
Penicillium sp.	5	2.1 a
	C	1.6 abcd
Fusarium oxysporum	7	1.6 abcd
Epicoccum sp.	A	1.3 cd
	B	1.6 abcd
Bacillus pumilus	D.1	1.8 abc
	D.T	1.3 cd
	FSII	1.5 abcd
P. phaseoli	P	1.9 ab

[x] Spore concentration is equal to 5,000 spores per ml. The temperature was 24 C.

[y] Means are from three repetitions, five plates per repetition, and 10 spores per replication.

[z] Numbers followed by the same letter are not significantly different from each other under Duncan's Multiple Range Test (P=0.05).

germination of spores in culture filtrate of isolates 3, A, and D.1 were significantly less than the P. phaseoli control filtrate. Spore germination was increased in the filtrate of Penicillium sp., isolate 5, nearly 21 percent.

Hyperparasitism by Fungal Antagonists

Hyperparasitism of Phomopsis was not observed with any of the potential antagonists. Hyperparasitism was observed only in the Rhizoctonia/Trichoderma control. Trichoderma hyphae within 7 days grew together, along side, and coiled around the hyphae of Rhizoctonia. In several areas, the coiling Trichoderma hyphae would grow into the Rhizoctonia hyphae. This did not occur between the antagonists and P. phaseoli. When the hyphae of the antagonists and P. phaseoli met, the hypha of both were observed to grow over each other and in some cases intertwine.

CHAPTER IV

DISCUSSION

All studies conducted were *in vitro* and are a prerequisite for further testing of potential inhibitors. *Fusarium oxysporum* and *Phomopsis* sp. lack potential for being considered control agents of *P. phaseoli*.

Changing temperatures did have an effect on the size of zones of inhibition produced between isolates and *P. phaseoli*. Considering all isolates, zones of inhibition were significantly larger at 22 and 24 C. Fungi and bacteria produced the smallest zones of inhibition at 26 C. Isolate 5, *Penicillium* sp., produced the greatest potential of all fungal isolates tested. Isolate 5 produced more spores between 22 and 32 C. *Epicoccum* isolates A and B produced significantly larger zones of inhibition at 24 and 28 C. Isolate C, *Penicillium* sp., is a rapid spore producer, whose spores are easily disseminated at all temperatures. *Epicoccum* isolates A and B and *Penicillium* sp. C have potential as biocontrol agents. All *B. pumilus* isolates produced thicker colonies as opposed to colonies larger in diameter as temperatures increased. This may account for the larger zones at 28-32 C. Wood (77) presented similar results when he challenged *Botrytis cinerea* with bacteria on lettuce.

Zones of inhibition produced by all isolates were significantly larger when carbon or nitrogen was absent or when carbon was deficient in a Modified Fries medium. Zones of inhibition produced by antagonists suggest that a diffusable substance inhibited Phomopsis. Bacterial isolates from the phylloplane produced significantly larger zones when carbon was absent or deficient. B. pumilus isolated from the seed, FSII, produced large zones when nitrogen was absent. Organisms that are stressed by the lack of specific nutrients can produce special metabolites which induce competition with pathogens (12,19). Wood (77) discovered when lettuce was sprayed with a 1 percent glucose solution, biocontrol organisms were able to compete with Botrytis cinerea.

Fewer Phomopsis spores germinated at a concentration of 5,000 per ml. Significantly fewer Phomopsis spores germinated when placed in the filtrates of isolates 3 (Monocillium), A (Epicoccum), and D.T (Bacillus pumilus). Some spores produce autolytic substances when they germinate (52). It is unknown if Phomopsis spores produce such a substance. Nutrients and temperature were optimum for Phomopsis spore germination. If produced, only isolate extracellular components could inhibit spore germination. Although germ tube elongation was inhibited, lysis was not observed in any of the Phompsis spores when challenged with

any isolate filtrates.

All B. pumilus isolates studied produced spores rapidly, increased in colony growth as temperatures increased, grew and competed on media in which carbon or nitrogen was absent, and produced spores which were viable after 5 years in test tube storage. During greenhouse tests, isolate FSII, did not reduce seed germination, cause necrosis of plant surfaces, or reduce plant vigor. B. subtilis, a related race of B. pumilus, has caused such reductions (16). Isolate FSII inhibited Phomopsis more efficiently than the other B. pumilus isolates in cross culture testing. Zones of inhibition were larger for isolate D.T when carbon was deficient or absent; however, isolate FSII produced larger zones throughout the C/N study. B. pumilus isolates also increased in growth and spore production as temperatures increased from 28 C to 32 C, temperatures which P. phaseoli invades and grows rapidly within the soybean (59).

B. pumilus has been suggested as a biocontrol agent for other plant pathogens. Morgan (52) reported that the filtrate of B. pumilus caused the uredospores of Puccinia spp. to lyse even after autoclaving the filtrate. The optimum temperature for lysing was 15 C. Any temperature above 27 C was determined to cause autolysing of the uredospores. When autoclaved cultures of B. pumilus

were applied to plant surfaces, the cultures did not inhibit germination of spores. Gayed (23) studied the effect of B. pumilus on Helminthosporium sativum spores when both were mixed. H. sativum spores lysed, suggesting that B. pumilus produces a lysing agent. The isolates of the bacterium vastly vary which may explain why a lysing of the Phomopsis spores did not occur in these tests.

B. pumilus could compete with Phomopsis from the beginning of the reproductive stage to seed harvest based on temperature results. B. pumilus is a regularly occurring bacterium on the soybean phylloplane, rhizosphere, and seed pods and should be able to compete even if carbon and nitrogen are stressed. This bacterium is nonpathogenic on barley and wheat (23), and from greenhouse tests nonpathogenic on soybean. Wood (77) suggested that due to changing environmental conditions field applications of bacterial biocontrol agents would need to be repeated. B. pumilus, if applied to seed, may colonize and protect the roots (10). B. pumilus has potential as a biocontrol agent of P. phaseoli. The filtrate of B. pumilus may be applied on leaf surfaces, instead of viable spores, based on the observations of Morgan (52).

Further research needs to be made on Diaporthe phaseolorum var. caulivora and Phomopsis phaseoli in areas such as infection courts, varying races, and toxin

production. The prominent biocontrols, *B. pumilus* isolates should be tested further. These tests include methods and timings of application to plants, the determination of their growth and survival on plant surfaces following application, their effect on other microflora, the effect of natural ultraviolet light on the inhibitors, and further *in vitro* testing to determine what metabolites are produced, by the inhibitors when tested with *P. phaseoli*.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Athow, K. L. and R. M. Caldwell. 1954. A comparative study of Diaporthe stem canker and pod and stem blight of soybean. Phytopathology 44:319-325.

2. Backman, P. A., M. Crawford, D. Weaver, and B. Cooper. 1983. Stem canker in Alabama: The effect of varieties and foliar sprays. Proc. South. Soy. Dis. Wor. 10:90-91.

3. Backman, P. A., D. Weaver, and G. Morgan-Jones. 1985. Soybean stem canker: An emerging disease problem. Plant Dis. 69:641-647.

4. Baker, R. 1985. Biological control of plant pathogens: Definitions. Pages 25-39 in Biological Control in Agriculture IPM Systems. M. A. Hoy and D. C. Herzog, eds. Academic Press, New York. 600 pp.

5. Baker, R. and F. M. Scher. 1987. Enhancing the activity of biocontrol agents. Pages 1-17 in Innovative Approaches to Plant Disease Control. I. Chet, ed. Wiley and Sons, Inc., New York. 372 pp.

6. Balducci, A. J. and D. C. Mc Gee. 1987. Environmental factors influencing infection of soybean seeds by Phomopsis and Diaporthe species during seed maturation. Plant Dis. 71:209-12.

7. Burra, L. R., J. P. Snow, and G. T. Berggren. 1986. Phytotoxin production by Diaporthe phaseolorum var. caulivora and its role in the stem canker disease of soybean. Phytopathology 76:1063. (Abstract).

8. Burra, L. R., J. P. Snow, and G. T. Berggren. 1989. Phytotoxin production by Diaporthe phaseolorum var. caulivora, the causal organism of stem canker of soybean. Phytopathology 79:499-504.

9. Chambers, A. Y. and M. A. Newman. 1988. Screening of soybean cultivars for stem canker resistance, 1982-87. Proc. South. Soy. Wor. 15:24. (Abstract).

10. Chao, W. L., E. B. Nelson, G. E. Harman, and H. C. Hoch. 1986. Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76:60-65.

11. Cook, R. J. and K. F. Baker. 1983. Antagonistae vitae. Pages 312-389 in The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Soc., Saint Paul. 539 pp.

12. Corke, A. T. K. and J. Rishbeth. 1981. Use of microorganisms to control plant diseases. Pages 717-736 in Microbial Control of Pests and Plant Diseases 70-80. H. D. Burgess. Academic Press, London. 949 pp.

13. Crall, J. M. 1950. Soybean diseases in Iowa in 1949. Plant Dis. Rep. 34:96-97.

14. Crall, J. M. 1952. A toothpick method of inoculation. Phytopathology 42:5-6. (Abstract).

15. Crall, J. M. 1956. Observations on the occurrence of soybean stem canker. Phytopathology 46:10. (Abstract).

16. Cubeta, M. A., G. L. Hartman, and J. B. Sinclair. 1985. Interaction between Bacillus subtilis and fungi associated with soybean seeds. Plant Dis. 69:506-509.

17. Dhingra O. D. and J. B. Sinclair. 1985. Basic Plant Pathology Methods. CRC Press, Boca Raton 355 pp.

18. Ebben, M. H. and D. M. Spencer. 1978. The use of antagonistic organisms for the control of black root rot of cucumber, Phomopsis sclerotioides. Ann. Appl. Biol. 89:103-106.

19. Elad, Y. and I. Chet. 1987. Possible role of competition for nutrients in biocontrol of Pythium damping-off by bacteria. Phytopathology 77:190-195.

20. Fravel, D. R. and H. W. Spurr Jr. 1977. Biocontrol of tobacco brown-spot disease by Bacillus cereus subsp. mycoides in a controlled environment. Phytopathology 67:930-932.

21. Frosheiser, F. I. 1957. Studies on the etiology and epidemiology of Diaporthe phaseolorum var. caulivora, the cause of stem canker of soybeans. Phytopathology 47:87-94.

22. Frosheiser, F. I. and M. F. Kernkamp. 1954. Asexual spore production in Diaporthe phaseolorum var. batatatis. Phytopathology 44:489. (Abstract).

23. Gayed, S. K. 1964. Bacillus pumilus and its mycolytic action against Helminthosporium sativum. Plant and Soil 24:178-180.

24. Gazaway, W. 1984. Stem canker in southeastern United States. Proc. South. Soy. Dis. Wor. 11:50. (Abstract).

25. Gershey, J. S., G. T. Berggren, Jr., and M. E. Pace. 1984. Timings of foliar fungicide applications on soybeans in Louisiana. Proc. South. Soy. Wor. 11:51. (Abstract).

26. Gindrat, D., E. van der Hoeven, and A. R. Moody. 1977. Control of Phomopsis sclerotioides with Gliocladium roseum or Trichoderma. Neth. J. Pl. Path. 83:429-438.

27. Hadar, Y., G. E. Harman, and A. C. Taylor. 1984. Evaluation of Trichoderma koningii and Trichoderma harzianum from New York soils for biological control of seed rot caused by Pythium spp. or Rhizoctonia solani. Phytopathology 74:106-110.

28. Hicks, J. R., L. J. Tomes, and D. M. TeKrony. 1983. Pod and seed infection by Phomopsis sp. during soybean seed development and maturation. Proc. South. Soy. Wor. 10:36. (Abstract).

29. Higley, P. M. and H. Tachibana. 1984. Resistance to stem canker of soybean and pathogenic specialization of the causal organism, Diaporthe phaseolorum var. caulivora. Phytopathology 74:1269. (Abstract).

30. Higley, P. M. and H. Tachibana. 1987. Physiologic specialization of Diaporthe phaseolorum var. caulivora in soybean. Plant Dis. 71:815-817.

31. Hildebrand, A. A. 1952. Stem canker: A disease of increasing importance on soybeans in Ontario. Soybean Digest 12:12-15.

32. Hildebrand, A. A. 1954. Observations on the occurrence of the stem canker and pod and stem blight fungi on mature stems of soybean. Plant Dis. Rep. 38:640-646.

33. Hilty, J. W. 1981. Soybean stem canker, a major disease in 1981. Tenn. Home and Farm Sci. 120:16-17.

34. Hilty, J. W. 1989. Pathogenic variation of isolates of the fungus causing soybean stem canker in Tennessee. Tenn. Home and Farm Sci. In press.

35. Hirrel, M. C. 1986. First report of stem canker on soybeans in Arkansas. Plant Dis. 70:78

36. Ilyas, M. B., O. D. Dhingra, M. Ellis, J. B. Sinclair. 1975. Location of mycelium of Diaporthe phaseolorum var. sojae and Cercospora kikuchii in infected soybean seeds. Plant Dis. Rep. 59:17-19.

37. Keeling, B. L. 1986. Effect of temperature, on the growth of Northern and Southern isolates of Diaporthe phaseolorum var. caulivora. Phytopathology 76:1115. (Abstract).

38. Keeling, B. L. 1988. Measurement of soybean resistance to stem canker caused by Diaporthe phaseolorum var. caulivora. Plant Dis. 72:217-220.

39. Keeling, B. L. 1988. Influence of temperature on growth and pathogenicity of geographic isolates of Diaporthe phaseolorum var. caulivora. Plant Dis. 72:220-222.

40. Kmetz, K., C.W. Ellett, and A. F. Schmitthenner. 1974. Isolation of seedborne Diaporthe phaseolorum and Phomopsis from immature soybean plants. Plant Dis. Rep. 58:978-982.

41. Kmetz, K. T., C. W. Ellett, and A. F. Schmitthenner. 1979. Soybean seed decay: Sources of inoculum and nature of infection. Phytopathology 69:798-801.

42. Kmetz, K. T., A. F. Schmitthenner, and C. W. Ellett. 1978. Soybean seed decay: Prevalence of infection and symptom expression caused by Phomopsis sp., Diaporthe phaseolorum var. sojae, and Diaporthe phaseolorum var. caulivora. Phytopathology 68:836-840.

43. Krausz, J. P. and B. A. Fortnum. 1983. An epiphytotic of Diaporthe stem canker of soybean in South Carolina. Plant Dis. 67:1128-1129.

44. Kulick, M. M. 1988. Observations by scanning electron and bright field microscopy on the mode of penetration of soybean seedlings by Phomopsis phaseoli. Plant Dis. 72:115-118.

45. Lehman, S. G. 1922. Pod and stem blight of soybeans. J. Elisha Mitchell. Sci. Soc. 38:13.

46. Lehman, S. G. 1923. Pod and stem blight if soybeans. Ann. Missouri Bot. Gard. 10:111-178.

47. Lifshitz, R., M. T. Windham, and R. Baker. 1986. Mechanism of biological control of preemergence damping- off of pea by seed treatment with Trichoderma spp. Phytopathology 76:720-725.

48. Luttrell, E. S. 1947. Diaporthe phaseolorum var. sojae on crop plants. Phytopathology 37:445-465.

49. Mc Gee, D. C. 1983. Epidemiology and control of soybean seed diseases. Proc. South. Soy. Dis. Wor. 10:69. (Abstract).

50. Mc Gee, D. C. 1983. Epidemiology of soybean seed decay by Phomopsis and Diaporthe spp. Seed Sci. and Tech. 11:719-729.

51. Moody A. R. and D. Gindrat. 1977. Biological control of cucumber black root rot by Gliocladium roseum. Phytopathology 67:1159-1162.

52. Morgan, F. L. 1963. Infection inhibition and germ tube lysis of three cereal rusts by Bacillus pumilus. Phytopathology 53:1346-1348.

53. Morgan-Jones, G. and P. A. Backman. 1984. Characterizaion of southeastern biotypes of Diaporthe phaseolorum var. caulivora the causal organism of soybean stem canker. Phytopathology 74:815. (Abstract).

54. Morgan-Jones, G. 1985. The Diaporthe/Phomopsis complex on soybean: Morphology. In Proceedings of the Conference on the Diaporthe/Phomopsis Disease Complex on Soybean. USDA, Agr. Res. Serv. pp.1-7.

55. Mulrooney, R. P. 1988. Southern United States soybean disease loss estimate for 1987. Proc. South. Soy. Dis. Wor. 15:33-37.

56. Peterson, J. L. and R. F. Strelecki. 1965. The effect of variants of Diaporthe phaseolorum on soybean germination and growth in New Jersey. Plant Dis. Rep. 49:228-229.

57. Ploetz, R. C. and F. M. Shokes. 1985. Soybean stem canker incited by ascospores and conidia of the fungus causing the disease in the southern United States. Plant Dis. 69:990-992.

58. Ploetz, R. C. and F. M. Shokes. 1986. Influence of temperature, inoculum density, and cultivar susceptibility on the infection of soybean seedlings by southern Diaporthe phaseolorum. Phytopathology 76:1082. (Abstract).

59. Ploetz, R. C. and F. M. Shokes. 1987. Factors influencing infection of soybean seedlings by southern Diaporthe phaseolorum. Phytopathology 77:786-790.

60. Ploetz, R. C. and F. M. Shokes. 1987. Infection of different plant parts of soybean seedlings by southern Diaporthe phaseolorum and its role in the department of stem canker symptoms. Can. J. Bot. 65:2104-2108.

61. Rothrock, C. S., T. W. Hobbs, and D. Phillips. 1985. Effects of tillage and cropping system on incidence and severity of southern stem canker of soybeans. Phytopathology 75:1156-1159.

62. Rothrock, C. S., D. V. Phillips, and T. W. Hobbs. 1988. Effects of cultivar, tillage, and cropping system on infection of soybean by Diaporthe phaseolorum var. caulivora and southern stem canker symptom development. Phytopathology 78:266-270.

63. Roy, K. W. and W. A. Miller. 1983. Soybean stem canker incited by isolates of Diaporthe and Phomopsis spp. from cotton in Mississippi. Plant Dis. 67:135-137.

64. Rupe, J. C. and R. S. Ferriss. 1986. Effects of pod moisture on soybean seed infection by Phomopsis spp. Phytopathology 76:273-277.

65. Russin, J. S. and D. J. Boethel. 1986. Effects of girdling by the three cornered Alfalfa Hopper on symptom expression of soybean stem canker and associated soybean yields. Plant Dis. 70:759-761.

66. Russin, J. S., M. B. Layton, D. J. Boethel, E. C. Mc Gawley, J. P. Snow, and G. T. Berggren. 1988. Severity of soybean stem canker disease in response to insect-induced defoliation. Proc. South. Soy. Wor. 15:26. (Abstract).

67. Russin, J. S., D. B. Orr, M. B. Layton, and D. J. Boethel. 1988. Incidence of microorganisms in soybean seeds damaged by stink bug feeding. Phytopathology 78:306-310.

68. Sinclair, J. B. 1977. The microcosm of the soybean seed. Ill. Research 19:12-13.

69. Sinclair, J. B., Ed. 1982. Compendium of Soybean Diseases. American Phytopathological Society, St. Paul, Minn. pp.39-40; 85-87.

70. Sleesman, J. P. and C. Leben. 1976. Microbial antagonists of Bipolaris maydis. Phytopathology 66:1214-1218.

71. Smith, E. F., P. A. Backman, and M. A. Crawford. 1986. Epidemiology of Diaporthe phaseolorum var. caulivora and stem canker development in southern soybeans. Phytopathology 76:1094. (Abstract).

72. Sundheim, L. 1977. Attempts at biological control of Phomopsis sclerotioides in cucumber. Neth. J. Pl. Path. 83:439-442.

73. TeKrony, D. M., D. Egli, J. Balles, L. Tomes, and R. E. Stuckey. 1984. Effect of date of harvest maturity on soybean seed quality and Phomopsis sp. seed infection. Crop Sci. 24:189-193.

74. Wehmeyer, L. E. 1933. The genus Diaporthe Nitsche and its segregates. Univ. Mich. Press, Ann Arbor, Mich. pp.1-349.

75. Welch, A. W. and J. C. Gilman. 1948. Hetero- and homo-thallic types of Diaporthe on soybeans. Phytopathology 38:628-637.

76. Wolf, F. A. and S. G. Lehman. 1920. Notes on new or little known plant diseases in North Carolina in 1920. North Carolina Agr. Exp. Sta. Ann. Rep. 43:55-58.

77. Wood, R. K. S. 1951. The control of diseases of lettuce by the use of antagonistic organisms. Ann. Appl. Biol. 38:203-216.

78. Yeh, C. C. and J. B. Sinclair. 1980. Effect of Chaetonium cupreum on seed germination and antagonism to other seedborne fungi of soybean. Plant Dis. 64:468-470.

VITA

Tamera Dawn Klingbyll was born in Nashville, Tennessee on November 24, 1964. She attended grades one through five in that city. She attended grades six through twelve in Fairview, Tennessee, where her family had moved. She graduated from Fairview High School in May 1983. The following September she entered David Lipscomb College, and in June 1987 she received a Bachelor of Science degree in Biology. In August of that same year, she accepted a graduate research assistantship at The University of Tennessee, Knoxville and began study toward a Master's degree. This degree was awarded in May 1990.