FLUID DRILLING: A POTENTIAL DELIVERY SYSTEM FOR *TRICHODERMA* SPP. AS BIOCONTROL AGENTS

By

CHRISTINE GABRIELLE FISHER

Bachelor of Science

University of Delaware

Newark, Delaware

1976

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1984

FLUID DRILLING: A POTENTIAL DELIVERY SYSTEM FOR TRICHODERMA SPP. AS BIOCONTROL AGENTS

Thesis Approved:

Kenneth E. Conway
Thesis Adviser

L. L. Singleton

H. A. Melouk

James E. Motes

Norman N. Durham
Dean of the Graduate College

PREFACE

This study evaluated the efficacy of delivering a biological control agent, *Trichoderma harzianum*, in a fluid drilling system. After isolating and identifying a native *T. harzianum* which was antagonistic to *Rhizoctonia solani*, the antagonist was evaluated for its ability to control damping-off of chile peppers.

The author wishes to express her appreciation to her major adviser, Dr. Kenneth E. Conway, for his guidance and assistance throughout the study. Appreciation is also expressed to the other committee members, Dr. H. A. Melouk, Dr. J. E. Motes, and Dr. L. L. Singleton for their assistance during my research and in preparation of the final manuscript.

I am also thankful to Mr. Bruce Bostian, supervisor of the Oklahoma Vegetable Research Station, Bixby, OK, for his help in my field studies and sage advice. Special thanks to Dr. Larry Claypool for assistance and processing of statistics. I am thankful to Bryan Brown and all the student workers, particularly Zahra Bahrani, for their help in the field and processing of soil samples. Special thanks to Steve Tomasino for his friendship, encouragement, and assistance.

Appreciation is extended to my parents, Mr. and Mrs. Ronald F. Bowden, for their inspiration and moral support through the years. A special thanks to my brother, Dr. Ed Bowden, who encouraged me to pursue a graduate degree, and has supported me throughout my studies.

A final expression of gratitude goes to my husband, Kim, who has been a constant source of support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Research involving biological control of soilborne plant pathogens has received much attention during the past ten years. Most current research utilizes the genus Trichoderma (form-class Deuteromycetes) which is a common soil-inhibiting saprophyte.

In 1972, Wells et al. (63) found Trichoderma harzianum Rifai present in sclerotia of Sclerotium rolfsii Sacc. and used this isolate to reduce S. rolfsii damage in tomatoes in greenhouse and field studies. Others isolated additional strains of T. harzianum from soil which were antagonistic to S. rolfsii and Rhizoctonia solani Kuhn (15,30,33,38).

Although T. harzianum is a potential biological control agent under experimental conditions, there are problems associated with its commercial use. A variety of carriers have been developed for delivering the biocontrol agent to soil, but all are commercially impractical because of excessive amounts of carrier required for application (3,38,63). However, findings by Harman et al (40,41) using T. hamatum (Bon.) Bain as a seed treatment may eliminate this problem. They also found that T. hamatum was a superior antagonist compared to T. harzianum for control of

disease caused by Pythium spp. and R. solani.

Another area of investigation is the application of fluid drilling or gel seeding technology to biological control problems. This technique involves the addition of germinated seeds to a gel carrier with subsequent extrusion into soil. The major advantage of sowing germinated seed compared to dry seed is earlier and more uniform emergence (21). The gel protects the exposed radical from mechanical damage and also provides the growing seedling with an initial water source. Unfortunately, the gel tends to attract microorganisms, including soilborne pathogens, which may result in an increased incidence of disease. Conway et al. (19) have used fungicides as adjuvants to the gel matrix to decrease damping-off disease caused by R. solani in chile peppers. Likewise, Ohep et al. (47) have added fungicides to gel to control Pythium aphanidermatum in tomatoes, and Entwistle (33) added ipridione to control white rot in onions. Fluid drilling offers what seems to be an ideal system for delivery of a biological control agent such as Trichoderma for control of soilborne disease problems.

Thus, this research had three major objectives: 1) Isolate and identify a strain of Trichoderma which was antagonistic to a pathogenic isolate of Rhizoctonia solani; 2) Study the efficacy of delivering the antagonist in a fluid drilling system in a controlled environment and in the field; and 3)Determine populations of the pathogen and the antagonist over time in a field situation.

CHAPTER II

REVIEW OF THE LITERATURE

Fluid Drilling

Fluid drilling is a technique in which germinated seeds are suspended in a gel matrix and then extruded into the soil. The system, also known as gel seeding, was originally developed in England by Elliott (32) for reseeding grasslands. Research in fluid drilling has continued in England, with most of the work being done at the National Research Station in Wellsbourne (12,23,36,56). Interest in fluid drilling in the United States has continued to grow as evidenced by increased horticultural research and greater commercial acceptance (34,59). At present large commercial acreages of tomatoes are being fluid drilled in Florida. The major advantages of using germinated seeds compared to dry seeds are earlier and more uniform emergence (21).

Examining the procedure in more detail, seeds are germinated in temperature controlled, aerated water to encourage uniform development of the embryo. Controlling the temperature of germination allows some flexibility in soil temperature for planting. As an example, tomato seeds require a high germination temperature, but can grow once

germinated at a lower temperature (12). After the radicals emerge, germinated seeds can be hand-separated from non-germinated seeds, if working on a small scale. In bulk quantities, germinated seed can be separated from non-germinated seed based on their different relative densities (60).

Drilling of the seeding gel can be done in a variety of ways. For small plantings, a plastic bag with the corner clipped off is used to extrude the gel. Additionally, caulking guns and hand pushed gear-driven seeders can be used in small plots. On a commercial scale, tractor mounted extrusion drills are used to pump the gel into the soil.

The Antagonist

The genus Trichoderma is a common soil fungus rarely known to cause plant disease. When cited as a plant pathogen it has only been associated with storage diseases (20).

Trichoderma, form-class Deuteromycetes, is the imperfect state of the teleomorph Hypocrea. The fungus is a fast grower initially appearing hyaline. Microscopically one of its most distinguishing features is the presence of flask shaped phialides occurring at right angles to the conidiophores. Conidia are borne on the phialides and may be either hyaline or more commonly green. The conidia en masse are what give Trichoderma its typical green appearance when viewed macroscopically.

The first key to this group of fungi was developed by Gilman and Abbott (35) in 1927. This was the only key available until 1969 when Rifai (54) published his monograph. He distinguished nine different species based primarily on microscopic characteristics. Although Rifai's work is still considered the authoritative source on Trichoderma, a slightly expanded version was published by Domsch, Gams, and Anderson (25) in 1982. Included in this key are two additional species not previously differentiated by Rifai.

The Pathogen

Rhizoctonia solani is a ubiquitous fungus that causes many diseases on a wide variety of hosts. Diseases caused by this pathogen include root rots, stem cankers, fruit decay, foliage diseases, and damping off. Pre- and post-emergence damping-off are common diseases affecting many types of seedlings. Damping-off is a rotting of seedlings at or below the soil line. Pre-emergence damping-off occurs when a young plant is killed before it emerges from the soil. In contrast, post-emergence damping-off occurs when a seedling is attacked near the soil line after emergence, resulting in death and collapse.

Rhizoctonia solani is the imperfect state of the basidiomycete Thanatephorus cucumeris (Frank) Donk. Rhizoctonia solani is classified in the form-order Agonomycetales (often referred to as Mycelia Sterilia) which

is in the form-class Deuteromycetes (1). Over the years, the taxonomy and identification of R. solani has been confusing because the fungus produces no conidia. Short of inducing the perfect state, the fungus must be identified by its hyphal characteristics alone. In 1970 Parmeter and Whitney (53) published a summary of characteristics which they felt gave the greatest accuracy in identification of R. solani and would exclude many similar fungi.

Characteristics consistently present:

1. Multinucleate cells in young vegetative hyphae
2. Prominent septal pore apparatus (dolipore septum)
3. Branching near the distal septum of cells in young vegetative hyphae.
4. Constriction of the branch and formation of a septum in the branch near the point of origin.
5. Some shade of brown.

Characteristics usually present:

1. Monilioid cells (swollen hyphal cells)
2. Sclerotia (without differentiated rind and medulla)
3. Hyphae greater than 5 μm in diameter
4. Rapid growth rate
5. Pathogenicity

Characteristics never possessed:

1. Clamp connections
2. Conidia
3. Sclerotia differentiated into a rind and medulla
4. Rhizomorphs

5. Red, green, blue, bright yellow, orange, or other pigments, except brown.
6. Any perfect state other than T. cucumeris

Historically, anastomoses grouping has been used as a criterion for mating group identification (53). This technique involves hyphal contact between a known isolate of R. solani and an isolate suspected of being R. solani. Hyphal fusion of the two isolates gives a definitive identification of R. solani, and places it into one of five subgroups. Unfortunately, failure to anastomose (or fuse) is not an adequate basis for excluding the isolate from truly being R. solani. Thus, while positive anastamoses gives definitive proof of species identification, lack of fusion is at best inconclusive.

The Use of Trichoderma as a Biocontrol Agent of Rhizoctonia solani

The earliest reference to the use of Trichoderma as a biocontrol agent was in 1932 by Weindling (62). He observed that Trichoderma lignorum (Tode) Harz was capable of parasitizing a strain of R. solani, a pathogen known to cause damping-off of citrus seedlings. He suggested the addition of the T. lignorum as a protective action during the period in which the citrus seedlings were most susceptible to damping-off. In 1935, Allen and Haenseler (2) confirmed Weindling's findings and also isolated a diffusible toxic compound from the Trichoderma which they

proposed was responsible for its activity. Although in 1964, Webster and Lomas (61) identified Weindling's T. lignorum as a Gliocladium sp., its importance in the Trichoderma literature should remain, since the two genera are similar.

Daines (22) working with potato [1937], noted the antagonistic action of Trichoderma sp. on R. solani in one out of three different soil types. Deines didn't pursue the work any further because he felt that the physical environment was too great a barrier against establishment of Trichoderma in the soil.

In 1956, Boosalis (11) noted that R. solani was parasitized by Trichoderma sp. and Penicillium vermiculatum. Unfortunately, he only pursued the work utilizing P. vermiculatum.

In their book, Ecology of Soil-borne Plant Pathogens, K.F. Baker and W.C. Snyder (5) summarized all that was known about biological control to that point in time [1965]. They made little mention of the Trichoderma-Rhizoctonia interaction owing to the paucity of references available at that time.

Research using Trichoderma started anew in the late 1960's and early 1970's. Initially the work centered on control of southern blight caused by Sclerotium rolfsii. Rodriguez-Kabana (55) utilized an isolate of Trichoderma viride to control southern blight of tomatoes under greenhouse conditions.

In the early 1970's, Wells et al (63) found T. harzianum present in diseased sclerotia of S. rolfsii. They successfully used this isolate to reduce southern blight damage in tomatoes under greenhouse and more importantly, field conditions.

In 1971, Dennis and Webster (24) published a rather lengthy article dealing with the hyphal interactions between Trichoderma isolates and various test fungi, including R. solani They observed both physical interactions, i.e., coiling, and the production of volatile and non-volatile antibiotics.

Baker and Cook (4) published Biological Control of Plant Pathogens in 1974. Although this book might have been the catalyst needed to encourage others to work in the biological control area, it actually contains few references to Trichoderma-Rhizoctonia interactions.

Studies conducted by Backman and Rodriguez-Kabana (3) in 1975 utilized the antagonist T. harzianum to control southern blight of peanut. They applied Trichoderma on diatomaceous earth granules impregnated with molasses. This was considered to be the first economical method for delivery of a biocontrol agent under field conditions.

During the late 1970's and early 1980's, four major groups of researchers utilized Trichoderma to control R. solani.

The first group of researchers were Israeli scientists who had initially worked with R. solani inoculum density

studies during the mid-1960's (42,43,57). In 1978, Henis, Ghaffer, and Baker (a cooperating U.S. researcher) (44) published their first article dealing with biocontrol. In that study, T. harzianum was applied using a wheat bran carrier to protect radish seedings from R. solani-induced damping-off. In addition, pentachloronitrobenzene (PCNB) (4ug/g soil), used in conjunction with T. harzianum, had an additive effect on disease control and decreased the inoculum density of R. solani.

The next year, Hadar, Chet and Henis (38) isolated a native T. harzianum from soil which was an antagonist to R. solani. Their disease control studies were conducted exclusively in the green house using a wheat bran carrier, for delivery of the antagonist to the soil. They also measured quantities of the lytic enzymes, B-(1-3) glucanase and chitinase, produced by the Trichoderma isolate on various carbon sources. Expanding this study in 1982, they found that various isolates of T. harzianum differed in the levels of hydrolytic enzymes produced when they attacked the mycelia of S. rolfsii, R. solani, and P. aphanidermatum (29). Correlations were found between the different enzyme levels and the ability of the isolate to control disease caused by the respective soilborne pathogen.

Chet, Hadar, Elad, Katan, and Henis (15) isolated a second T. harzianum capable of attacking R. solani and S. rolfsii. Field work in S. rolfsii-infested soils showed a small but significant decrease in the percentage of diseased

plants (56.3% diseased in control plots vs. 43.1% in the T. harzianum-treated plots.)

Their continued interest in the interaction between pesticides and Trichoderma prompted a study on the interaction between dinitroaniline herbicide and T. harzianum to control S. rolfsii on peanuts. They found no benefit in applying the herbicide and biocontrol agent together (37).

Working with Baker again in 1980, they determined that the method of antagonism for T. harzianum appeared to be a parasitism, since no antibiotic activity could be detected (13). This was also the first attempt to quantify propagules of R. solani over time in a monoculture system. They found that soil became suppressive to R. solani with successive croppings and that the suppressiveness was accompanied by an increase in propagule density of T. harzianum. In a later study, they noted that a soil conducive to R. solani could be transformed into a suppressive soil by the addition of a T. hamatum isolate (14). The T. hamatum isolate had been previously isolated from a soil known to be suppressive to R. solani.

Continuing their interest in an integrated approach to biological control, Elad et al (31) utilized solarization (polyethylene mulching), fumigants (methyl bromide and vapam) PCNB, and T.harzianum. Solar heating or fumigation with methyl bromide, along with T. harzianum improved the efficiency of using either treatment alone.

Another new isolate of T. harzianum recovered from soil was found to significantly decrease disease caused by S. rolfsii and R. solani (30). This biocontrol organism was more efficient in controlling disease at lower temperatures which corresponded with in vitro laboratory temperature studies. An interesting observation from this study was that T. harzianum increased plant growth, particularly height. This would be a distinct advantage over pesticides which frequently cause phytotoxicity.

In 1981, Chet et al. (16) published photographs of the interaction between R. solani and T. hamatum. Using phase contrast and Nomarski differential interference-contrast microscopy, it appeared that the Trichoderma hyphae produced appressorial structures or hooked-shaped contact branches. The Trichoderma hyphae then penetrated the Rhizoctonia hyphae and grew within, eventually lysing the host cells. Additional observations of these interactions were made in 1982 using electron and fluorescent microscopy (27).

The second group to work with Trichoderma-Rhizoctonia systems was Ralph Baker and associates from Colorado State University. Prior to working with the Trichoderma-Rhizoctonia system, Ralph Baker's major interest centered on the epidemiology and modelling of R. solani (6-10). Baker and co-workers (45) developed a soil sampler used for quantitative estimation of propagules of R. solani in soil. Although he co-authored many of the previously discussed papers, only one major paper dealing with this system came

out of his lab. Liu and Baker (46) found that suppressiveness built-up in soil after monoculture with radish or cucumber. An increase in the number of Trichoderma propagules correlated with a decrease in the inoculum density of R. solani. In addition, they induced suppressiveness in a conducive soil by the addition of Trichoderma.

The third group of researchers is located at Geneva, N.Y. and is directed by Gary Harmon. In 1980, Harmon, Chet, and Baker (40) determined the feasibility of using T. hamatum [later to be re-identified as T. harzianum (39)] as a seed treatment to protect seeds and seedlings from attack by Pythium spp. and R. solani. On pea and radish seeds, T. hamatum provided disease control equal to captan and PCNB fungicide treatments in greenhouse studies. The activity of the T. hamatum continued providing disease control through two additional plantings.

Following-up their first study, Harmon et al. (41) examined the addition of amendments to T. hamatum seed treatments. The addition of chitin or cell walls of R. solani to seed coats increased the ability of T. hamatum to protect seeds against Pythium spp. and R. solani. The amended seed treatments also resulted in an increase in the population density of Trichoderma in the soil, probably due to a readily available food source.

The fourth major researcher involved in this biocontrol system is George Papavizas of the Soilborne Diseases

Laboratory, Beltsville, MD. After many years of examining survival of R. solani in soil, Papavizas(48,50,51) began a program using ultra-violet radiation induced biotypes of T. harzianum. In 1982, Papavizas, Lewis, and Abd-El Moity (52) reported on the development of new biotypes of T. harzianum that were selected for their resistance to benomyl, enhanced biocontrol capabilities, and ability to survive in soil. Several UV-induced biotypes were consistently more effective in suppressing disease caused by R. solani and P. ultimum than the wild strain.

CHAPTER III

MATERIALS AND METHODS

1982 Field Study

Field studies were performed at Bixby and Stillwater, Oklahoma. Studies at Bixby took place in three different field sites at the Oklahoma Vegetable Research Station. At Stillwater, one field site was utilized at the Plant Pathology Farm.

Eight treatments were evaluated using a randomized complete block design with four replications. Treatments consisted of two different strains of T. harzianum, Strain 1 (St-1) (courtesy of R. Baker) and Strain 2 (St-2) (courtesy of Abbott Labs) incorporated into Laponite 508 gel (synthetic magnesium silicate) (Laporte (U. S.) Inc., Continental Plaza 411 Hackensack, NJ 07601) at two rates: 10^5 and 10^7 conidia/ml of gel. Conidial concentrations were adjusted using a hemacytometer. Additional treatments consisted of growing both T. harzianum strains on oats and incorporating the infested oats into the soil at time of planting. The last two treatments were an unamended control gel, and a gel containing the fungicide captan (100 ug active ingredient (a.i.)/ml. Planting rows were 3.8 m long

and 0.9 m apart. Germinated Bahamian Hot Chile seeds were added to the gel at the rate of 164 seeds/100 ml of gel. Gel was extruded into naturally infested the soil using either a modified hand push drill or caulking guns at the rate of 20 ml of gel/m.

Soil samples were taken at 3-4 weeks after planting. Soil samples were made from within the planting row to a depth of 10 cm using a cork borer 13 mm in diameter. Soil was serially diluted with sterile deionized water (1:100, 1:1000, 1:10,000) and then 1 ml of each dilution was plated onto Trichoderma Selective Medium (TSM) (28). Plates were incubated in the dark at room temperature (25 C) for 7 days before making colony counts.

Isolation of Antagonist from Soil

A 9 cm circle of nylon net (1 mm mesh) was autoclaved (121 C, 1.05 kg/cm^2) and placed on the surface of potato dextrose agar (PDA) in a petri dish. A plug of Rhizoctonia solani (AG-4) (R-96) (7 mm) was then placed on top of the net and allowed to grow to the edge of the petri dish. The nylon net, with R. solani mycelium attached, was peeled off the agar and cut into 1 cm squares. The squares were equally distributed between two 9 cm glass petri dishes containing 100 g of soil "suppressive" to R. solani (Conway, unpublished). One dish of soil was incubated for 24 hours and the other one for 7 days. After the incubation period, soil was placed on a sieve and washed under running water

for 3 min to recover the nylon net squares. Nylon squares were plated onto either PDA amended with 300 mg/L streptomycin sulfate or Trichoderma selective medium (TME) (49). Trichoderma-like fungi growing from the nylon squares and onto the media were transferred to PDA for further study.

Screening of Potential Antagonists on Artificial Medium

Five mm plugs were cut from actively growing cultures of R. solani (R-96) on PDA, and potential antagonists selected from the previous isolation procedure. Plugs of R. solani and a potential antagonist were placed 3 cm apart on sterile glass microscope slides covered with 2.5 ml of 2% water agar. Paired-culture slides were incubated in petri dishes until cultures met. Slides were placed on a compound microscope and interactions between hyphae observed. Aniline blue dye was used to stain the hyphae.

Identification of the Potential Antagonist

Initial microscopic observation indicated that the fungus was a Trichoderma sp. and further species identification followed. The fungus was grown on malt agar in 9 cm petri dishes and on microscope slides each covered with 2.5 ml of malt agar and placed in a petri dish. Both types of cultures were incubated in the dark for 48 hours and then exposed to 12 hours of light. Colonies were examined for

characteristic features. Cultures were sent to J. Bissett at the Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario K1A 0C6 for confirmation.

Optimum Growth Temperatures of *Trichoderma harzianum* and *Rhizoctonia solani* in Culture

Agar disks (7 mm) covered with mycelium of *R. solani* (R-96) or *Trichoderma harzianum* (T-224) were transferred from the edge of three day old colonies to the center of PDA contained in 9 cm petri dishes. Colonies were incubated in darkness at temperatures ranging from 10 C to 35 C, at 5 C increments. Colony diameters were measured daily for five days or until the mycelia reached the edge of the petri dish. There were four replications and the experiment was repeated once.

Inoculum Density of *Rhizoctonia solani* in Cornmeal Sand Culture

A 4% cornmeal sand mixture (CMS) (8 g cornmeal, 196 g sand, and 50 ml deionized water) was placed in 250 ml Erlenmeyer flasks, cotton stoppered, and autoclaved for 1 hr on two consecutive days (121 C, 1.05 kg/cm^2, 60 min). A plug (9 mm) was cut from an actively growing agar culture of *R. solani* (R-96) and added to each flask. A piece of aluminum foil was crimped over each cotton stopper to retard moisture loss. The inoculated CMS flasks were incubated in darkness

at 20 C. One flask was sacrificed each week for quantitation of viable propagules. CMS was diluted using a sterile soil mix before pelleting. A multiple pellet soil sampler (45) was used to determine propagule density. There were four replications per sample. Counts were made at 24-36 hours using a dissecting microscope. Corrections were made for multiple pellet colonization (45). The experiment was repeated two times and run for a minumum of seven weeks.

Germination Technique

Bahamian Hot Chile seeds (Capsicum annuum l.) (courtesy of J.E. Motes) were used in all experiments. Seeds were germinated in a temperature controlled germinator, until radicals were exposed to a length of 1-3 mm. The germinator consisted of a plexiglass chamber which contained fitted glass cylinders, 4.5 cm diameter and 40 cm long (58). Cylinders were rubber stoppered at the bottoms. The chamber and glass cylinders were filled with tap water. Aeration was provided by an aquarium pump connected by tubing to an air stone located at the bottom of each tube. Water temperature was maintained at 27 C by the use of an aquarium heater. Water was changed daily for four days or until the seeds were removed. Unless otherwise indicated, germinated seeds were hand-separated from non-germinated seed.

Environmental Chamber Studies

General

All plantings were made in aluminum pans 6 cm X 3 cm X 3 cm, which contained 120 g soil. Soil was a pasteurized mix of 1 part sandy loam soil: 2 parts sand: 1 part peat moss and was adjusted to 10% moisture content before planting. Fifteen germinated chile seeds suspended in gel were planted 2-3 mm deep in 3 rows in each container of soil at the rate of 15 seeds/3 ml of gel. Gel consisted of powdered Laponite 508 blended with water at the rate of 1.6% w/v. After seeds were planted, pans were loosely covered with plastic wrap to reduce evaporation. Temperature was maintained at 25 C with a 12 hour light-dark cycle. There were four replications per treatment.

Inoculum of *R. solani* (R-96) was produced on 4% CMS. After incubation for 3-4 weeks at 25 C, the CMS inoculum was added at the rate of 2% by weight to soil. Soil was infested 24 hours prior to planting. This resulted in 8-20 *R. solani* propagules/g of soil at time of planting.

Disease Control by the Addition of *Trichoderma harzianum* to Soil

Trichoderma harzianum (T-224) conidia were harvested from 20 day old cultures on PDA by adding 3 ml of sterile water to the agar surface and then dislodging the conidia with a rubber spatula. Conidial concentrations were

determined with a hemacytometer. The conidia were added at the rate of 10^7 conidia/g of soil infested with R. solani. The augmented soil was incubated at 25 C for 3 days prior to planting. Emergence and damping-off were recorded at 7 days after planting. Comparisons were made between R. solani-infested treatments and R. solani-T. harzianum-infested treatments.

In a later study T. harzianum was grown on sterile oats (equal quantities of oats and water autoclaved at 1.05 kg/cm^2 for 45 minutes) for one week prior to planting. Infested oats were added to the top 5 mm of soil at the rate of 1.2 grams per pan of soil just prior to planting. Disease control due to the addition of T. harzianum-infested oats was compared to a captan amended gel treatment.

Amendments to Gel

Conidia of T. harzianum (T-224) were harvested from 2-3 week old cultures on PDA. The agar surface was flooded with 3 ml of sterile deionized water and the conidia were rubbed free with a rubber spatula. Conidial concentrations were determined using a hemacytometer and then adjusted to desired concentrations. Three concentrations of conidia were used: 10^7, 10^8, and 10^9 conidia/ml of gel. Viability of T. harzianum conidia in gel was determined (18). Captan (100 ug a.i./ml) was used as a comparison of disease control. The experiment was repeated once.

1983 Field Study

Field studies were performed at the Plant Pathology Farm, Stillwater, Oklahoma. All plantings were made in raised bed microplots with an interior measuring 2.3 X 2.3 m. Soil was a sandy loam, pH 5.7-6.3, which had been fumigated with methyl bromide 4 weeks prior to planting. Treatments were laid out in a randomized complete block design. Each microplot contained four rows 2.2 m long and 0.6 m apart. Treatments were located in the two center rows and were 2 m in length. The two outer rows as well as the remaining 0.1 m at the end of each treatment row were unamended guard rows.

Rhizoctonia solani (R-96) grown on CMS was added to half of the microplots at the rate of 2% w/w of soil, just prior to planting. CMS (1920 g sand: 80 g cornmeal: 500 ml deionized water) was placed into 2.8 L Fernback flasks, autoclaved, and then infested with ten 9 mm plugs of a 3 day old culture of R. solani. The flasks filled with infested CMS were incubated for nine days prior to planting. Infested CMS was hand-mixed into the planting row to a width of 15 cm and a depth of 8 cm. Germinated Bahamian Hot Chile seeds were gel seeded into the soil at the rate of 66 seeds/ 40 ml gel (Laponite 508, 1.6% w/v)/ 2 m row.

Gel treatments consisted of an unamended control, T. harzianum (T-224) at the rate of 10^7 conidia/ml of gel, and captan at 100 ug a.i./ml. Trichoderma conidia were

harvested from 20 day old PDA plates by adding 3 ml of sterile water to the agar surface and then dislodging the conidia with a rubber spatula. Conidial concentrations were determined using a hemacytometer and then adjusted to the desired concentration.

Application of the gel was made using small plastic bags with the corner clipped off. After the gel was extruded, it was covered with 1-2 mm of soil and lightly irrigated.

Rhizoctonia solani and Trichoderma propagule counts from microplot soil were made pre-plant and at 5 day intervals for 20 days. Soil samples were taken using a 12 mm cork borer to a depth of 4 cm. Four samples were taken 1-1.5 cm from the gel treatment and then bulked. Counts of R. solani propagules were made using a multiple pellet soil sampler (45), and placing the soil pellets on a selective inulin-copper sulfate medium (IN) (26). There were four replications per soil sample. Plates were incubated in the light at 25 C for 48 hours before reading. Counts were made using a dissecting microscope at 15-105 X. Trichoderma propagule counts were made by serially diluting soil with sterile water(1:100, 1:1000, 1:10,000) and then delivering 1 ml of each dilution onto four replicate plates of a Trichoderma selective medium(TME) (49). Plates were incubated at 25 C in the light for 8-10 days before colony counts were made. Seedling emergence counts were made every 2-3 days.

CHAPTER IV

RESULTS

1982 Field Study

Fluid drilling studies were performed at Bixby and Stillwater, Oklahoma. Trichoderma propagule counts were made from planting row soil 3-4 weeks after gel seeding of germinated chile seeds. Multiple rank transformations were made on all data (17). Significantly (P=0.05) higher densities of Trichoderma propagules were recovered from soil samples of treatments where Trichoderma spp. were incorporated either on oats or in gel at 10^7 conidia/ml (Table 1).

Screening of Potential Antagonist on Artificial Medium

Microscopic examination of the interaction of the hyphae of R. solani (R-96) and T. harzianum (T-224) revealed several types of associations (Figures 1, 2, and 3). Hyphae of T. harzianum coiled around R. solani hyphae (Figure 1), or moved in a serpentine fashion along the surface of R. solani hyphae (Figure 2). T. harzianum also produced curved infection hooks or pegs into the hyphae of R. solani (Figure 3).

TABLE 1. Mean rank of *Trichoderma* propagules from planting row soil 3-4 weeks after incorporation with germinated chile seeds, 1982.

Treatment	Mean Rank of *Trichoderma* Propagules			
	Location			
	Bixby 1	Bixby 2	Bixby 3	Stillwater
T. harzianum (St-2)				
Gel(10^5 conidia/ml)	10.0(7.6)ab[x,y]	8.2(3.5)a	8.0(8.3)a	9.4(5.9)ab
Gel(10^7 conidia/ml)	22.5(19.4)cd	20.2(47.5)b	18.5(50.8)b	26.0(30.6)de
Oats	27.0(31.4)d	----	----	30.5(54.8)e
T. harzianum (St-1)				
Gel(10^5 conidia/ml)	3.9(4.7)a	11.2(4.6)a	8.4(8.9)a	12.0(6.4)abc
Gel(10^7 conidia/ml)	16.2(15.0)bc	20.2(46.5)b	----	20.0(13.8)cd
Oats	26.8(40.4)d	----	----	16.8(7.8)bc
Captan	13.4(7.8)b	6.9(3.0)a	10.5(9.6)a	10.5(5.7)ab
Control[z]	12.2(8.2)ab	7.6(3.3)a	7.1(8.8)a	6.9(4.4)a

[x]Values in parenthesis are average numbers of *Trichoderma* propagules($X\ 10^{-2}$)g dry soil.

[y]Mean ranks in the same column followed by the same letter are not significantly different according to Duncan's multiple range test, $P \_ 0.05$.

[z]Captan (100 ug a.i./ml gel).

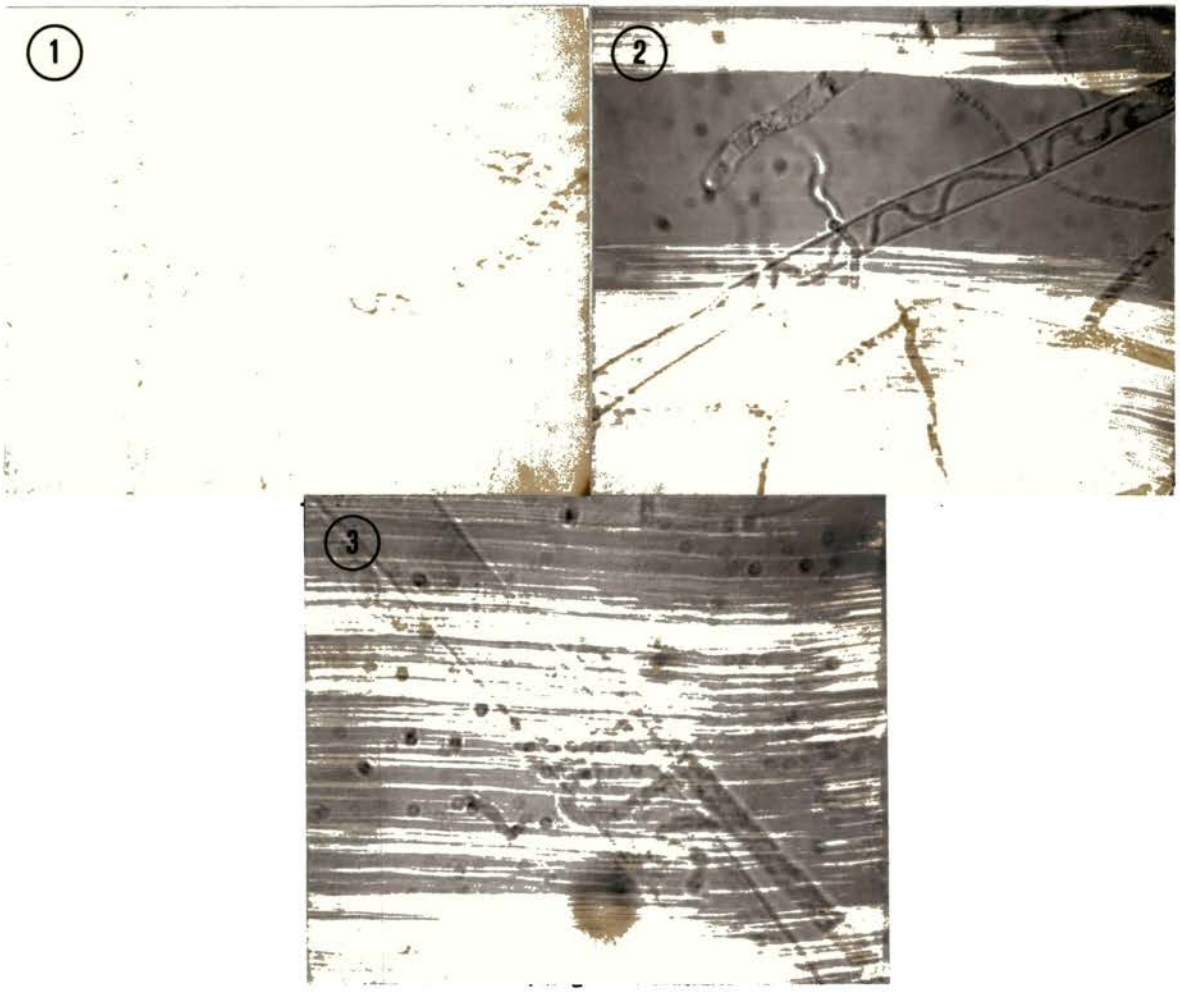

Figure 1. Trichoderma harzianum coiling around R. solani hyphae.

Figure 2. Serpentine movement of T. harzianum along surface of R. solani.

Figure 3. Infection hooks or pegs formed by T. harzianum.

Identification of the Potential Antagonist

The fungus used in antagonistic studies was examined from pure culture using the procedure of Rifai (52). Conidia were smooth-walled, and globose to obovoid, 3-3.5 X 3-4 um. Phialides were rather narrow bottle shapes with long necks (8-12 X 2-2.5 um) (Figure 4). Phialides arose in false verticils of two to four beneath the terminal phialide. There were no sterile hyphal elongations. The main branches of the conidiophores produced many right-angled side branches (Figure 5). The complex branching of the conidiophores resulted in a pyramidal or conical pattern. When grown in agar culture, the mature colony had a reverse coloration of dark golden-yellow.

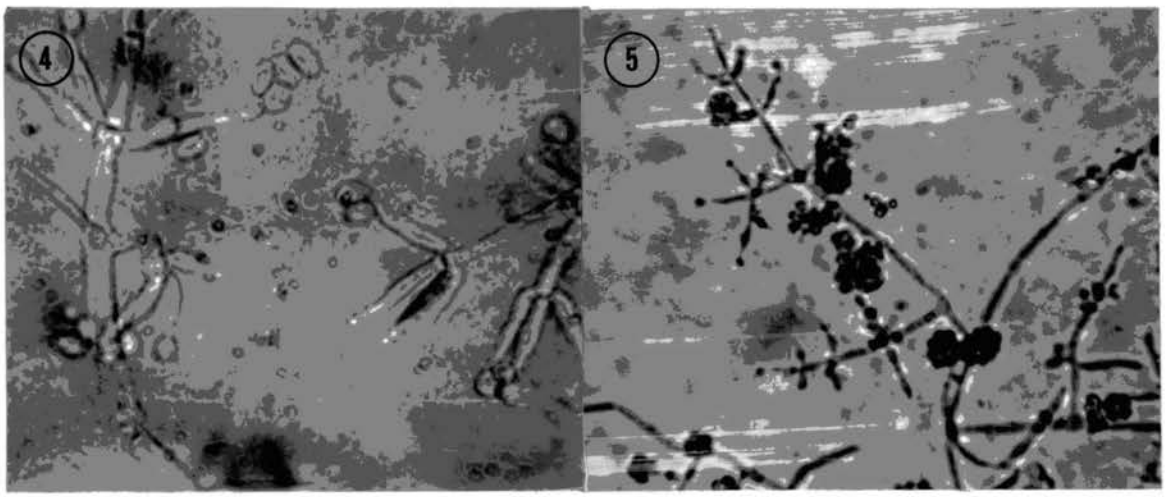

Figure 4. Trichoderma harzianum conidia and phialides (1000 X).

Figure 5. Typical branching pattern of T. harzianum (400 X).

The culture was initially identified as Trichoderma aureoviride, but was later identified by J. Bissett as T. harzianum, based on the size of the phialides and conidia.

Optimum Growth Temperatures of Trichoderma harzianum and Rhizoctonia solani in Culture

Diametral hyphal growth of T. harzianum (T-224) on PDA was greatest at 30 C (Figure 6) but it also grew well at 25 C. Rhizoctonia solani (R-96) grew well within a range of 20-30 C, with optimal growth at 25 C.

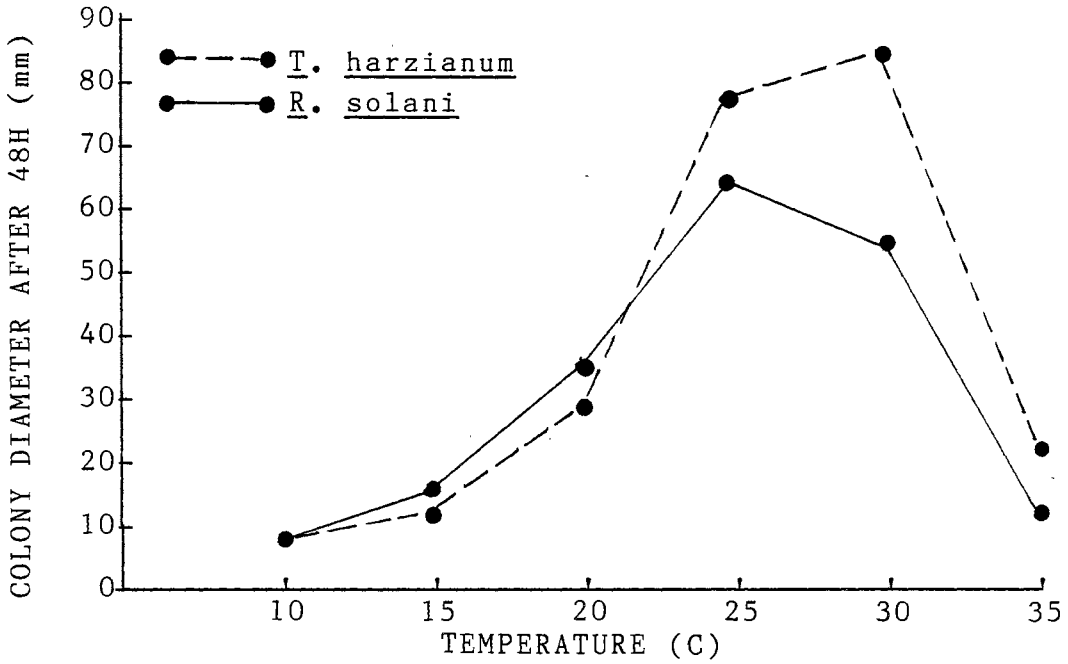

Figure 6. The effect of temperature on diametral growth of R. solani (96) and T. harzianum (224) on potato dextrose agar.

Inoculum Density of *Rhizoctonia solani* in Cornmeal Sand Culture

The quantity of *R. solani* (R-96) propagules present in the first three weeks varied widely (Figure 7). During this early period, propagule counts/g dry sand were relatively high in the first (269-409) and third (335-441) experiments but quite low in the second (34-77). After the third week, the number of propagules increased to more than 500 in all experiments. In the first experiment, the propagule number peaked at 995 during the seventh week. The second and third experiments peaked at the sixth and fifth weeks, respective-

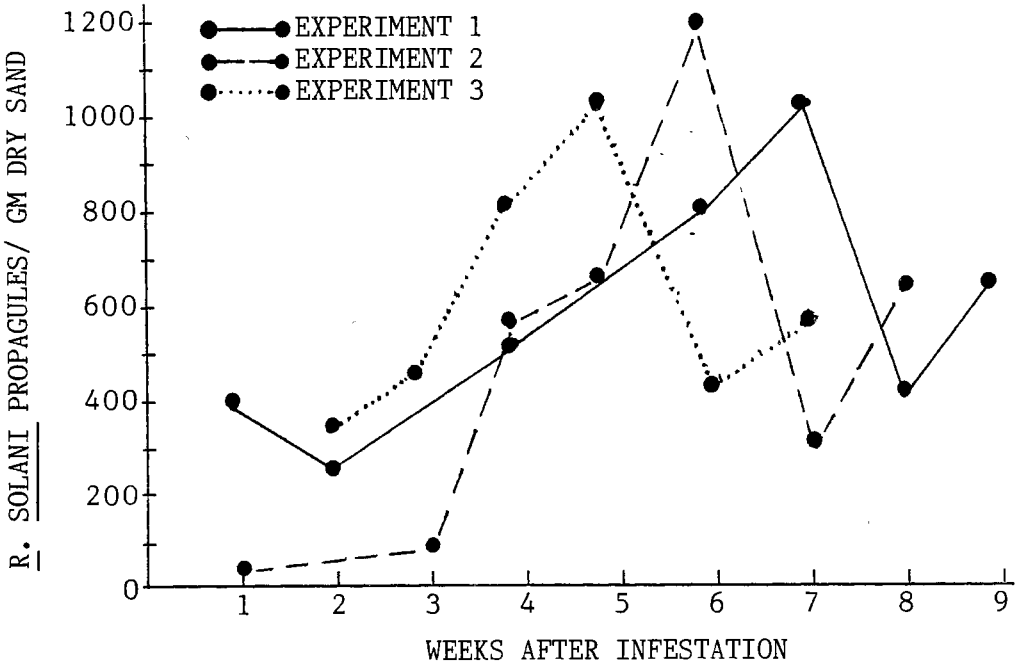

Figure 7. Inoculum density of *Rhizoctonia solani* (R-96) over time in a 4% cornmeal sand culture.

In all experiments, the number of viable propagules declined after the initial peak, but still remained high (>250 propagules/g dry sand) until the end of the experiment.

Environmental Chamber Studies

Disease Control by the Addition of *Trichoderma harzianum* to Soil

Trichoderma was added to soil using two different methods: conidia added directly to soil or on infested oat seeds. Germinated chile seeds, suspended in an unamended gel, were gel-seeded into soil. When conidia of *T. harzianum* (T-224) were added directly to the soil prior to gel-seeding, there was a significant (P=0.05) decrease in damping-off in only one experiment (Table 2). The decrease in damping-off, though significant was quite small (33%) when compared to the *R. solani* infested control.

In contrast, when *T. harzianum* was added to soil on an oat carrier prior to fluid drilling, there was a large and significant decrease in diseased plants when compared to the *R. solani* infested control (Table 3). The *T. harzianum* treatment resulted in a 57% decrease in disease in Experiment 1 and a 68% decrease in Experiment 2. An additional treatment using a captan amendment resulted in a 54% and 61% decrease in damping-off compared to the *R. solani* infested controls in Experiments 1 and 2, respectively.

TABLE 2. The effect of *Trichoderma harzianum* (224) added directly to soil as conidia, on the incidence of damping-off of chile seedlings caused by *Rhizoctonia solani*.

Treatment	Diseased Plants (%)		
	Exp 1	Exp 2	Exp 3
Uninfested Control	0 A[y]	0 A	0 A
R. solani Control	30 B	88 B	48 B
R. solani + *T. harzianum*[z]	20 AB	77 AB	32 C

[y]Numbers in each column followed by the same letter do not differ significantly according to Duncan's multiple range test, P=0.05.
[z]*Trichoderma harzianum* added at 10^7 conidia/g of soil.

TABLE 3. The effect of *T. harzianum* (T-224) added to soil on an oat carrier, on the incidence of damping-off of chile seedlings, caused by *R. solani*.

Treatment	Diseased Plants (%)	
	Exp 1	Exp 2
Uninfested Control	0 A[y]	0 A
R. solani Control	68 B	77 B
R. solani + *T. harzianum*	29 C	25 C
R. solani + captan[z]	31 C	30 C

[y]Numbers in each column followed by the same letter do not differ significantly from each other according to Duncan's multiple range test, P= 0.05.
[z]Captan (100 ug a. i./ ml gel).

Amendments to Gel

The effect of conidial densities of T. harzianum in the seeding gel, on damping-off was evaluated in two experiments. Trichoderma harzianum added at 10^9 conidia/ml gel, significantly decreased disease compared to the R. solani infested control in Experiment 1 only (Figure 8). All other densities of conidia failed to reduce disease. Addition of captan to the gel resulted in significantly better disease control (61% reduction) than the T. harzianum (10^9) treatment (27% reduction) (Table 4).

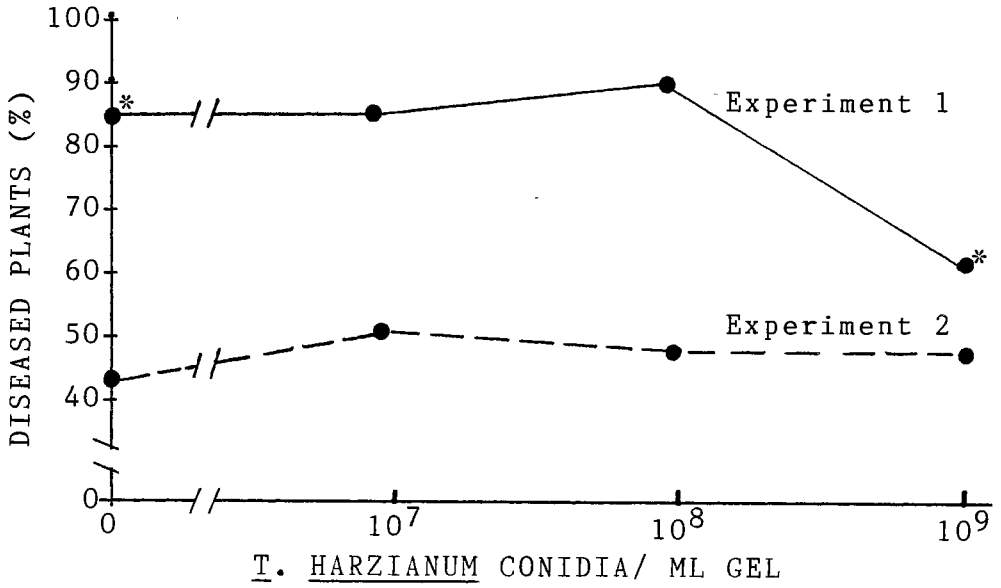

Figure 8. The effect of Trichoderma harzianum (224) at three different concentrations, on the incidence of damping-off of chile seedlings caused by Rhizoctonia solani. (*) indicates a significant difference P=0.05.

TABLE 4. The effect of T. harzianum (T-224), as an amendment to gel, on the incidence of damping-off of chile seedlings caused by R. solani.

Treatment	Diseased Plants (%)			
	Exp 1		Exp 2	
Uninfested Control	0	A[x]	0	A
R. solani Control	85	B	43	B
R. solani/ T. harzianum(10^7)	85	--[y]	52	--
R. solani/ T. harzianum(10^8)	90	--	48	--
R. solani/ T. harzianum(10^9)	62	C	47	B
R. solani/ captan[z]	33	D	27	B

[x]Numbers in each column followed by the same letter do not differ significantly from each other according to Duncan's multiple range test, P=0.05.
[y]Not included in the comparisons.
[z]Captan (100 ug a. i./ ml gel).

1983 Field Study

Gel seeding studies, using germinated chile seeds, were performed in raised-bed microplots at Stillwater, OK. Half of the microplots were infested with a CMS culture of R. solani (R-96). Three gel treatments were used in both the R. solani-infested and uninfested plots. Gel treatments consisted of an unamended gel, and gels amended with either T. harzianum (T-224) (10^7 conidia/ ml gel) or captan (100 ug a.i./ml gel). Multiple rank transformations were made on all data. Rhizoctonia solani propagules were monitored over time in the R. solani-infested microplots (Table 5). Rhizoctonia propagules decreased over time in all treatments from an initial density of 12 propagules/g soil to 3 propagules/g soil on the twentieth day.

TABLE 5. Mean densities of Rhizoctonia solani propagules/g dry soil over time, in microplots previously infested with R. solani CMS culture, Stillwater, 1983.

Treatment	R. solani Propagules/g Dry Soil				
	Days After Amendment				
	0	5	10	15	20
Control	12	33(8.7)[z]	5(7.8)	3(6.0)	3(7.8)
T. harzianum	12	34(9.3)	8(10.5)	9(13.3)	3(11.2)
Captan	12	38(10.5)	7(10.3)	5(9.2)	3(9.6)

[z]Number in parenthesis indicates mean rank.

There were no significant differences in mean rank of R. solani propagules among the unamended, T. harzianum or captan treatments on a given date (Figure 9).

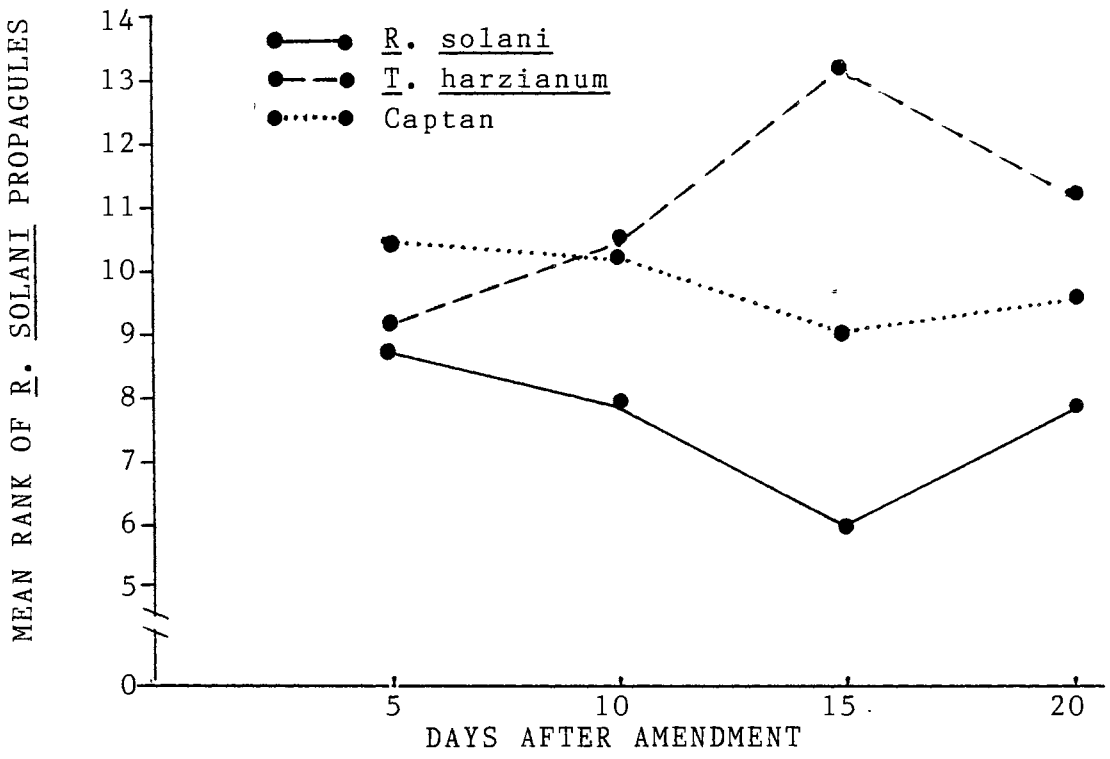

Figure 9. Mean rank of Rhizoctonia solani propagules/g dry soil over time in microplots, Stillwater, 1983.

Initial background counts of Trichoderma varied widely (Day 0-Table 6). Based on rank means, there were significantly more Trichoderma propagules present at 15 and 20 days in the R. solani infested-Trichoderma soil samples than in the uninfested-Trichoderma soil samples (Figure 10).

TABLE 6. Mean densities of *Trichoderma* propagules/g dry soil over time, in *Rhizoctonia solani*-infested and uninfested microplot soil, Stillwater, 1983.

Treatment	*Trichoderma* Propagules/g Dry Soil				
	Days After Amendment				
	0	5	10	15	20
Uninfested Soil					
Control	160(10.2)[x]	210(7.8)	550(9.5)	170(6.6)	1033(11.3)
T.harzianum[y]	700(13.2)	6783(15.8)	738(12.0)	972(9.9)	483(10.5)
Infested Soil[z]					
Control	1733(19.2)	3450(13.2)	1433(14.2)	1663(14.1)	667(8.7)
T.harzianum	43(7.5)	5277(13.3)	1660(14.3)	6567(19.4)	7367(19.6)

[x]Number in parenthesis indicates mean rank.

[y]*Trichoderma harzianum* fluid drilled into soil at the rate of 10^7 conidia/ml gel.

[z]Soil infested with CMS culture of *R. solani*.

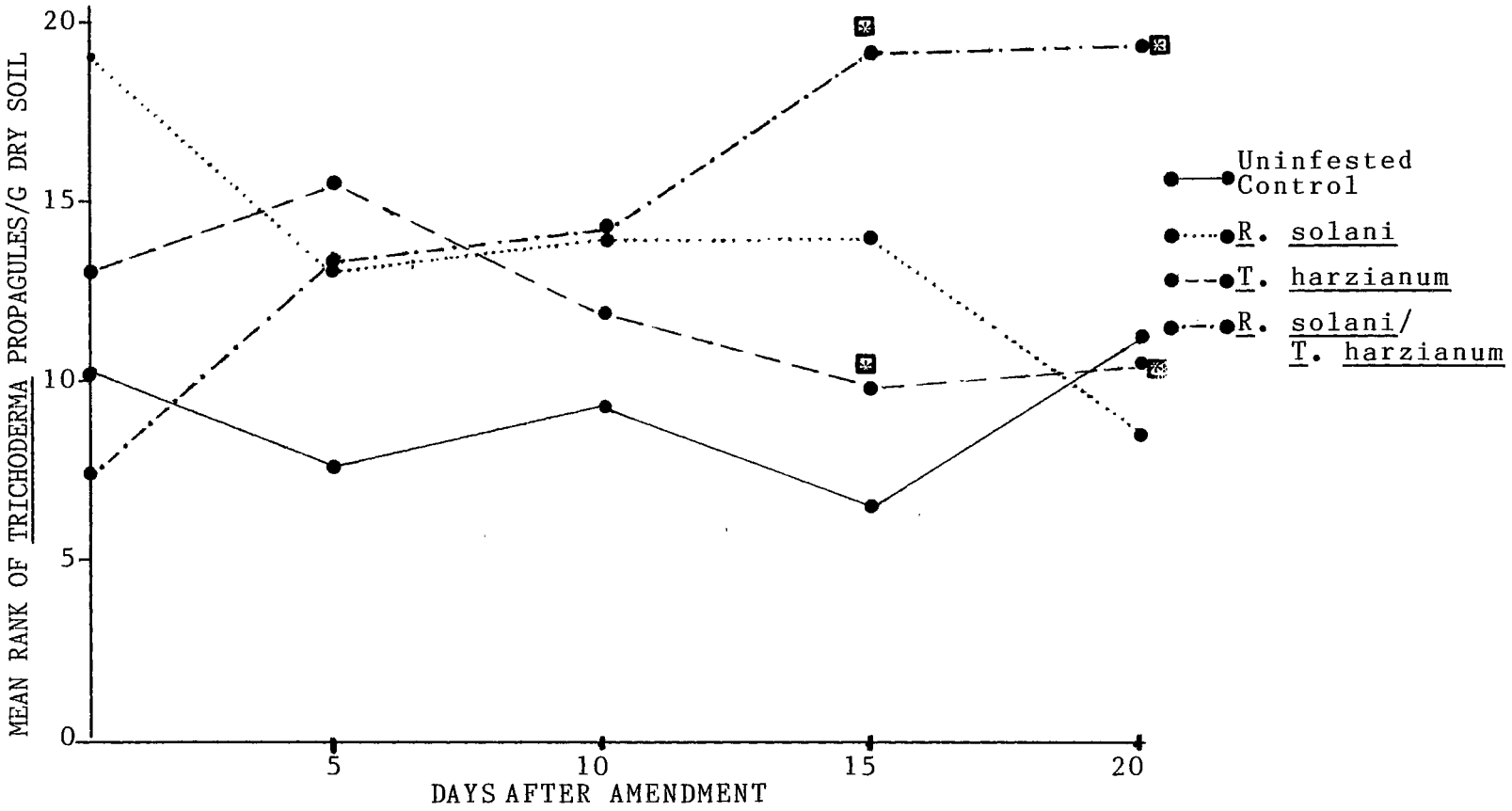

Figure 10. Mean ranks of Trichoderma propagules/g dry soil over time in microplot soil.(*)indicates a significant difference between contol and T. harzianum-infested plots based on the LSD P=0.05. Comparisons are within days.

Seedling emergence in the uninfested control plots averaged 69.7% (46 out of 66 seeds planted) while the emergence in the R. solani-infested plots averaged 10.6% (7 out of 66 seeds) (Table 7).

TABLE 7. Mean emergence of chile seedlings in Rhizoctonia solani-infested and uninfested microplots, Stillwater, 1983.

Treatments	Seedlings Emerged/ Row		
	Days After Plant		
	10	15	20
Uninfested Soil			
Control	40[x](28)[y]	50 (27)	46 (27)
Captan	43 (29)	52 (29)	51 (29)
T.harzianum	37 (26)	52 (27)	51 (29)
Infested Soil[z]			
Control	4 (10)	6 (7)	7 (6)
Captan	4 (9)	10 (10)	14 (13)
T.harzianum	3 (9)	11 (12)	11 (10)

[x]66 seeds were fluid drilled/ row.

[y]Number in parenthesis indicates mean rank.

[z]Soil infested with R. solani CMS culture.

There were no significant differences in plant emergence based on treatment (Figure 11). Rather the only significant difference in emergence was based on presence or absence of R. solani.

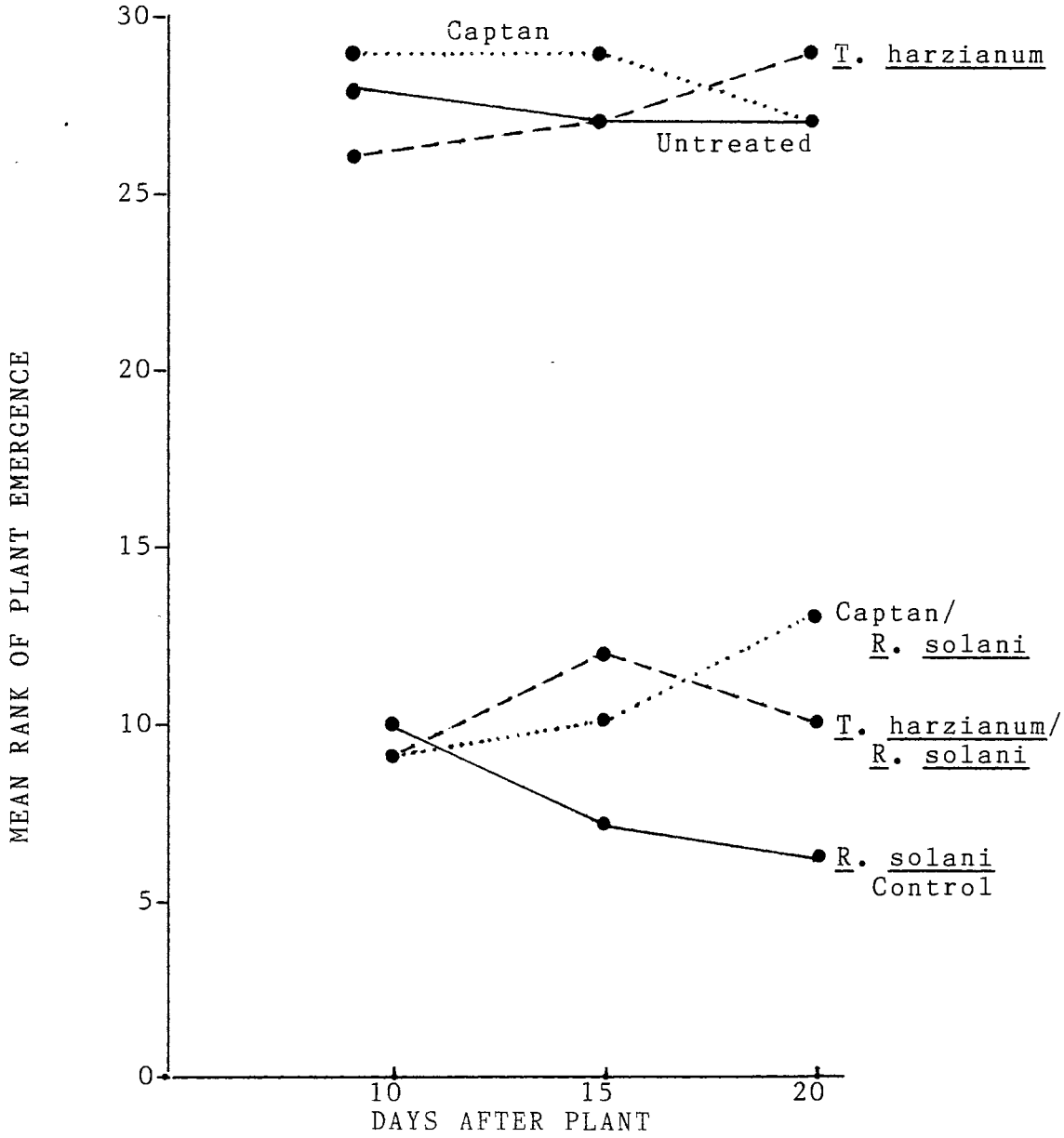

Figure 11. Mean ranks of chile seedling emergence over time in Rhizoctonia solani infested and uninfested microplots.

CHAPTER V

DISCUSSION

In 1982 field studies, two strains of *Trichoderma harzianum* were added to soil on an oat seed carrier or as conidia in gel. Although no seedling emergence data was available due to unfavorable environmental conditions, densities of *Trichoderma* propagules were determined at 3-4 weeks after planting. In most cases *Trichoderma* densities were significanty higher in 10^7 conidia/ml gel treatments than in the control. The densities were even higher in the oat seed treatments, probably due to the presence of a food base.

Further studies indicated that these two isolates of *T. harzianum* were not effective biocontrol agents for this disease system. Thus a baiting system was used to recover a native antagonistic *Trichoderma* sp..

Isolation of antagonistic *Trichoderma* spp. using baiting techniques is an accepted practice (46). Screening of potential antagonists on artificial media is a more controversial approach. *Trichoderma harzianum* isolated in these experiments proved to be an antagonist to *Rhizoctonia solani* on artificial medium and in soil. In culture, the antagonist coiled around and formed penetration hooks into

the R. solani hyphae. Vacuolization and dissolution of the R. solani hyphae also occurred. In environmental chamber studies, when oat seed infested with T. harzianum was used as a treatment, damping-off was reduced by an average of 57-68% compared to the infested control. George Papavizas and Laura Mihura (personal communications), would contend that this correlation between artificial media interactions and soil studies was mere coincidence. They have found no correlation between good antagonists on artificial media and in soil. Yet others (63) continue to screen antagonists exclusively on artificial media. In the view of this author future screenings of antagonists should be performed in soil.

Initial studies were made on the inoculum density of R. solani in CMS culture over time. In many studies researchers have grown R. solani on CMS for a set period of time and then mixed it into soil at a certain proportion. They have little idea of how many propagules were present. In this series of experiments, there was considerable variability in the number of propagules from experiment to experiment and over time. If the CMS culture had been used to infest soil during the first three weeks of storage, considerably fewer propagules would have been added than if an older culture had been used. This could make a significant difference in disease severity.

In environmental chamber studies, T. harzianum controlled damping-off only in the presence of a food base,

i.e. oats. When conidia were added directly to soil or into gel, there was no disease control, except in one experiment where the addition of 10^9 conidia/ml gel resulted in a significant decrease in damping-off. This is an extremely high concentration of conidia which would be impractical to use on a larger scale.

At no time in these studies did T. harzianum act as a pathogen of chile peppers. Although Elad et al. (30) reported an increased growth response in plants as a result of T. harzianum, no such effects were observed in these experiments.

In the 1983 Stillwater field study, R. solani added as a CMS culture apparently overwhelmed the system. Although the quantity of CMS culture added was based on previous environmental chamber studies, the concentration was too high for a field situation. As a result of this overkill situation the only significant difference in plant emergence occurred between non-infested microplots and those infested with R. solani. Although slightly more plants emerged in the T. harzianum and captan treatments than in the control plots, there were no significant differences.

There were also no significant differences in R. solani propagule densities among the control and T. harzianum or captan treatments. In contrast, there was a significant increase at P=0.05 in the density of Trichoderma propagules in the T. harzianum-R. solani plots over the T. harzianum-only plots at 15 and 20 days. One could hypothesize that

the increase in Trichoderma propagule density was the result of parasitism and utilization of R. solani. A more accurate explanation of this increase may be the presence of the cornmeal (present in the CMS) as a food source for the T. harzianum. As in the previous experiments, one can again see the use and need of a food base for the population growth of Trichoderma in the soil.

A possible follow-up to these studies would be the addition of a food base with the Trichoderma in the gel. Recent studies by Harman et al (41), have found promising results with the addition of various food sources to biological seed treatments.

A major problem that must be overcome in the addition of biological agents to gel is the time factor. When dry seed is added to soil, it takes time to germinate. During this time a biological seed treatment has time to germinate and colonize the seed coat. The actively growing antagonist would thus be able to protect the germinating seedling from surrounding pathogens. In contrast, in fluid drilling the radical is already exposed when planted. Although the seedling will emerge more quickly than in direct seeding, it is also immediately susceptible to R. solani damping-off. Therefore the gel must contain an actively growing biological agent along with the germinated seed at time of planting. Although this might be a possibility on a small research scale, this would not appear to be a commercially practical approach.

With pesticide residue and toxicity a problem in the environment, biological control is appealing as an alternative approach to plant disease problems. Unfortunately there are many inherent problems in its practical use. The biocontrol agent is a living organism, and like the pathogen it should control, has its own niche in the soil. Both organisms have a preference for various soil types, concentrations of nutrients and organic matter, water potentials, temperatures, and pH. These are just a few characteristics to consider. In addition, there are many different strains of R. solani, and although an antagonist may attack one or more of the strains, it may not attack all of them. Therefore, how does one come up with an antagonist that will control all strains of R. solani in all field situations? The answer at this point is unknown and stategies and techniques have not been formulated. For control of soil diseases, biological controls such as Trichoderma spp. will have to be used in specific areas. Since most of the successful biological control studies have taken place in controlled situations, such as growth chambers or greenhouses, the development of a biological control program for commercial greenhouses would be one such area. Rather than adding Trichoderma spp. to gel, a more promising situation might be the addition of Trichoderma spp. and a food source directly into a potting mix. In this way seedlings would be protected as they emerge and transplants would take their protective biological agents along

with them into their new soil environment. If gel seeding research using Trichoderma spp. is to be pursued, perhaps integrated control utilizing Trichoderma spp. and a fungicide would be the best approach. The fungicide would give immediate disease control, while the biocontrol could provide long-term disease protection.

LITERATURE CITED

1. Alexopoulos, C. J., and Mims, C. W. 1979.Introductory Mycology. John Wiley & Sons, New York. 632pp.
2. Allen, M. C., Haenseler, C. M. 1935. Antagonistic action of Trichoderma on Rhizoctonia and other soil fungi. Phytopathology 25:244-252.
3. Backman, P. A., and Rodriguez-Kabana, R. 1975. A system for the growth and delivery of biological control agents to the soil. Phytopathology 65:819-821.
4. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman & Co., San Francisco, CA. 433 pp.
5. Baker, K. F., and Snyder, W. C. 1965 Ecology of Soil-Borne Plant Pathogens. Univ. Calif. Press, Berkeley, CA. 571 pp.
6. Baker, R. 1971. Analyses involving inoculum density of soil-borne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
7. Baker, R., and Drury, R. 1981. Inoculum potential and soilborne pathogens: the essense of every model is within the frame. Phytopathology 71:363-372.
8. Benson, D. M., and Baker, R. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: influence of pentachloronitrobenzene. Phytopathology 64:38-40.
9. Benson, D. M., and Baker, R. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: inoculum potential interaction. Phytopathology 64:957-962.
10. Benson. D. M., and Baker, R. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: survival. Phytopathology 64:1163-1168.
11. Boosalis, M. G. 1956. Effect of soil temperature and green manure amendment of unsterilized soil on parasitism of Rhizoctonia solani by Penicillium vermiculatum and Trichoderma sp. Phytopathology 46:473-478.
12. Bussel, W. T., and Gray, D. 1976. Effects of presowing seed treatments and temperatures on tomato seed germination and seedling emergence. Scientia Horticulturae 5:101-109.
13. Chet. I., and Baker, R. 1980. Induction of supressiveness to Rhizoctonia solani in soil. Phytopathology 70:994-998.

14. Chet, I., and Baker, R. 1981. Isolation and biocontrol potential of Trichoderma hamatum from soil naturally suppressive to Rhizoctonia solani. Phytopathology 71:286-290.
15. Chet, I., Hadar, Y., Elad, J., and Henis, Y. 1979. Biological control of soil-borne plant pathogens by Trichoderma harzianum. Pages 585-592 in: Soil-Borne Plant Pathogens. B. Schippers and W. Gams, eds. Academic Press, New York. 686 pp.
16. Chet, I., Harman, G. E., and Baker, R. 1981. Trichoderma hamatum: its hyphal interactions with Rhizoctonia solani and Pythium spp. Microbial Ecol. 7:29-38.
17. Conover, W. J., and Iman, R. L. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. The American Statistician 35:124-129.
18. Conway, K. E. 1983. Viability of Trichoderma harzianum stored in fluid drilling gels at four temperatures. (Absta.) Phytopathology 73:820.
19. Conway, K. E., Fisher, C. G., and Motes, J. E. 1982. The use of fungicides in a seeding gel to prevent damping-off diseases. Proceedings from Okla. AgChem Conference 7:50-51.
20. Conway, W. S. 1983. Trichoderma harzianum: A possible cause of apple decay in storage. Plant Dis. 67:916-917.
21. Currah, I. E. 1978. Plant uniformity at harvest related to variation between emerging seedlings. Acta Hortic. 72:57-67.
22. Daines, R. H. 1937. Antagonistic action of Trichoderma on Actinomyces scabies and Rhizoctonia solani. The American Potato Journal 14:85-93.
23. Darby, R. J. 1978. Establishment of crops by fluid drilling. Pages 64-65 in: Report of the National Vegetable Research Station, Wellesbourne, UK.for 1977.
24. Dennis, C., and Webster, J. 1971. Antagonistic properties of species-groups of Trichoderma. III. Hyphal interaction. Trans. Br. Mycol. Soc. 57:363-369.
25. Domsch, K. H., Gams, W., and Anderson, T. H. 1980. Trichoderma. Pages 794-809 in: Compendium of Soil Fungi. Vol. 1. Academic Press, New York. 859 pp.
26. Doornik, A. W. 1980. Some factors affecting the parasitic and saprophytic activity of Rhizoctonia solani. Acta Hortic. 109:387-394.
27. Elad, Y., Chet, I., Boyle, P., and Henis, Y. 1983. Parasitism of Trichoderma spp. on Rhizoctonia solani and Sclerotium rolfsii- Scanning electron microscopy and fluorescence microscopy. Phytopathology 73:85-88.
28. Elad, Y., Chet, I., and Henis, Y. 1981. A selective medium for improving quantitative isolation of Trichoderma spp. from soil. Phytoparasitica 9:59-67.
29. Elad, Y., Chet, I., and Henis, Y. 1982. Degadation of plant pathogenic fungi by Trichoderma harzianum. Can. J. Microbiol. 28:719-725.

30. Elad, Y., Chet, I., and Katan, J. 1980. Trichoderma harzianum: A biocontrol agent effective against Sclerotium rolfsii and Rhizoctonia solani. Phytopathology 70:119-121.
31. Elad, Y., Katan, J., and Chet, I. 1980. Physical, biological, and chemical control integrated for soilborne diseases in potatoes. Phytopathology 70:418-422.
32. Elliott, J. G. 1966. The sowing of seeds in aqueous fluid. Pages 31-32 in: Report of the Weed Research Organization for 1965-66.
33. Entwistle, A. R., and Munnsinghe, H. L. 1981. Control of white rot (Sclerotium cepivorum) in fluid-drilled salad onions. Journal of Horticultural Science 56:251-254.
34. Gerber, J. 1981. Direct seeding tomatoes: the future is here. American Vegetable Grower 29:6-7.
35. Gilman, J. C., and Abbott, E. V. 1927. A summary of the soil fungi. Iowa St. Coll. J. Sci. 1:225-343.
36. Grimshaw, P. J. 1981. Fluid drilling: A British perspective. American Vegetable Grower 29:9-12.
37. Grinstein, A., Elad, Y., Katan, J., and Chet, I. 1979. Control of Sclerotium rolfsii by means of a herbicide and Trichoderma harzianum. Plant Dis. Rep. 63:823-826.
38. Hadar, 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.
39. Hadar, Y., Harman, G. E., and Taylor, A. G. 1984. Evaluation of Trichoderma koningii and T. harzianum from New York soils for biological control of seed rot caused by Pythium spp.. Phytopathology 74:106-110.
40. Harman, G. E., Chet, I., and Baker, R. 1980. Trichoderma hamatum effects on seed and seedling disease induced in radish and pea by Pythium spp. or Rhizoctonia solani. Phytopathology 70:1167-1172.
41. Harman, G. E., Chet, I., and Baker, R. 1981. Factors affecting Trichoderma hamatum applied to seeds as a biocontrol agent. Phytopathology 71:569-572.
42. Henis, Y. 1970. Significance of population level of Rhizoctonia solani in soil. Pages 34-36 in: Root Diseases and Soil-Borne Pathogens. T. A. Toussoun, R. V. Bega, and P. E. Nelson, eds. Univ. of Calif. Press, Berkeley,CA.
43. Henis, Y., and Ben-Yephet, Y. 1970. Effect of propagule size of Rhizoctonia solani on saprophytic growth, infectivity, and virulence on bean seedlings. Phytopathology 60:1351-1356.
44. Henis, Y., Ghaffer, A., and Baker, R. 1978. Integated control of Rhizoctonia solani damping-off of radish: effect of successive plantings, PCNB, and Trichoderma harzianum on pathogen and disease. Phytopathology 68:900-907.

45. Henis, Y., Ghaffer, A., Baker, R., and Gillespie, S. L. 1978. A new pellet soil-sampler and its use for the study of population dynamics of Rhizoctonia solani in soil. Phytopathology 68:371-376.
46. Liu, S., and Baker, R. 1980. Mechanism of biological control in soil supressive to Rhizoctonia solani. Phytopathology 70:404-412.
47. Ohep, J., McMillan, Jr., R. T., Bryan, H. H., and Cantliffe, D. J. 1984. Control of damping-off of tomatoes by incorporation of fungicides in direct-seeding gel. Plant Dis. 68:66-67.
48. Papavizas, G. C. 1969. Survival of root-infecting fungi in soil: XI. Survival of Rhizoctonia solani as affected by inoculum, concentration, and various soil amendments. Phtopathol. Z. 64:101-111.
49. Papavizas, G. C. 1982. Survival of Trichoderma harzianum in soil and in pea and bean rhizospheres. Phytopathology 72:121-125.
50. Papavizas, G. C., Adams, P. B., Lumsden, R. D., Lewis, J. A., Dow, R. L., Ayers, W. A., and Kantzes, J. G. 1975. Ecology and epidemiology of Rhizoctonia solani in field soil. Phytopathology 65:871-877.
51. Papavizas, G. C., and Davey, C. B. 1961. Saprophytic behavior of Rhizoctonia solani in soil. Phytopathology 51:693-699.
52. Papavizas, G. C., Lewis, J. A., and Abd-El Moity, T. H. 1982. Evaluation of new biotypes of Trichoderma harzianum for tolerance to benomyl and enhanced biocontrol capabilities. Phytopathology 72:126-132.
53. Parmeter, J. R., and Whitney, H. S. 1970. Taxonomy and nomenclature of the imperfect state. Pages 7-19 in: Rhizoctonia solani, Biology and Pathology. J. R. Parmeter, ed. Univ. of Calif. Press, Berkeley, CA. 255 pp.
54. Rifai, M. A. 1969. A revision of the genus Trichoderma. Commonw. Mycol.. Inst. Mycol. Pap. 116.
55. Rodriguez-Kabana, R., Kelley, W. D., and Curl, E. A. 1978. Proteolytic activity of Trichoderma viride in mixed culture with Sclerotium rolfsii. Can. J. Microbiol. 24:487-490.
56. Salter, P. J. 1978. Techniques and prospects for fluid drilling of vegetable crops. Acta Hortic. 72:101-108.
57. Sneh, B., Katan, J., Henis, Y., and Wahl, I. 1966. Methods for evaluating inoculum density of Rhizoctonia solani in naturally infested soil. Phytopathology 56:74-78.
58. Sosa-Coronel, J. 1981. Studies on seed germination and storage of germinated seed of different pepper (Capsicum sp.) cultivars- their applications in fluid drilling. Ph.D. dissertation, Oklahoma State University.
59. Taylor, A. G. 1977. Comparative performance of pregerminated, high moisture content, and dry vegetable seed in greenhouse and field studies. J. Seed Tech. 2:52-61.

60. Taylor, A. G., Motes, J. E., and Price, H. C. 1978. Separating germinated from ungerminated seed by specific gravity. Hortscience 13:481-482.
61. Webster, J., and Lomas, N. 1964. Does Trichoderma viride produce gliotoxin and viridin? Trans. Br. Mycol. Soc. 47:535-540.
62. Weindling, R. 1932. Trichoderma lignorum as a parasite of other fungi. Phytopathology 22:837-845.
63. Wells, H. D., Bell, D. K., and Jawarski, C. A. 1972. Efficacy of Trichoderma harzianum as a biological control for Sclerotium rolfsii. Phytopathology 62:442-447.

VITA 2

Christine Gabrielle Fisher

Candidate for the Degree of

Master of Science

Thesis: FLUID DRILLING: A POTENTIAL DELIVERY SYSTEM FOR TRICHODERMA SPP. AS BIOCONTROL AGENTS

Major Field: Plant Pathology

Biographical:

Personal Data: Born in Seaford, Delaware, April 12, 1954, the daughter of Marie A. and Ronald F. Bowden. Married to Kim N. Fisher on May 13, 1978.

Education: Graduated from Seaford Senior High School, Seaford, Delaware, in June, 1972; received Bachelor of Science Degree in Medical Technology from the University of Delaware in June, 1976; completed requirements for the Master of Science Degree at Oklahoma State University in December, 1984.

Professional Experience: Medical Technologist at Johnston-Willis Hospital, Richmond, VA, 1976-1981; Graduate Teaching Assistant, Department of Plant Pathology, Oklahoma State University, 1981-1982; Research Assistant, Department of Plant Pathology, Oklahoma State University, 1982-1983; Agricultural Technician IV, Department of Plant Pathology, Oklahoma State University, 1983-1984.

Professional Organizations: American Phytopathological Society; Southern Division of American Phytopathological Society; Oklahoma Academy of Science.

Honor Societies: Phi Kappa Phi.