PATHOGENICITY OF Bacillus penetrans
TO Meloidogyne incognita

BY

STEPHEN MICHAEL BROWN

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1983

to

Marie, Lora and Johanna

ACKNOWLEDGEMENTS

I express my sincere gratitude to my major professor, G. C. Smart, Jr., for guidance, encouragement and continued interest throughout the course of this study. I am grateful to D. W. Dickson, R. P. Esser, D. J. Mitchell and E. P. Previc for serving on my committee and for constructive review of my dissertation. I am most grateful to D. J. Mitchell for his friendship, for his innovative plant pathology course and for his considerable encouragement throughout my studies, and to J. L. Kepner for allowing me to attend his multivariate, nonparametric and regression courses in statistics, for use of an unpublished statistical test and for many helpful comments on the manuscript. I thank D. Nordmeyer for many suggestions about techniques, Nguyen Ba Khuong for Figure 3.1, P. C. O'Brien for help in initiating the field experiments, C. Baeza Aragón for collaboration on the sampling of nematodes in soil and E. B. Whitty for providing tobacco seedlings and agronomic advice.

This study was supported in part by a research assistantship from the Department of Entomology and Nematology and a United Brands student loan and research grant.

TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGEMENTS.	iii
LIST OF TABLES.	vi
LIST OF FIGURES	viii
ABSTRACT.	xi
INTRODUCTION.	1
CHAPTER 1: INCREASED CROP YIELDS FOLLOWING APPLICATION OF <u>Bacillus penetrans</u> TO PLOTS INFESTED WITH <u>Meloidogyne incognita</u>	6
Introduction	6
Materials and Methods.	7
Results.	12
Discussion	18
Summary.	20
CHAPTER 2: INCREASED CROP YIELDS FOLLOWING APPLICATION OF <u>Bacillus penetrans</u> TO PLOTS INFESTED WITH <u>B. penetrans</u> AND <u>Meloidogyne incognita</u>	22
Introduction	22
Materials and Methods.	22
Field Experiments	22
Determination of a Dosage-Response Function for the Inoculum of <u>Bacillus penetrans</u>	24
Attachment of Spores to <u>Meloidogyne javanica</u> , <u>M. incognita</u> and <u>M. arenaria</u>	25
Results.	27
Field Experiments	27
Determination of a Dosage-Response Function for the Inoculum.	29

	<u>Page</u>
Attachment of Spores to <u>Meloidogyne javanica</u> , <u>M. incognita</u> and <u>M. arenaria</u>	29
Discussion	32
Summary.	34
CHAPTER 3: EFFECT OF <u>Bacillus penetrans</u> ON MOVEMENT OF AND ROOT PENETRATION BY <u>Meloidogyne incognita</u>	35
Introduction	35
Materials and Methods.	35
Root Penetration by <u>Meloidogyne incognita</u> Infected with <u>Bacillus penetrans</u>	35
Movement of <u>Meloidogyne incognita</u> Infected with <u>Bacillus penetrans</u>	37
Results.	40
Discussion	43
Summary.	47
DISCUSSION AND CONCLUSIONS.	48
LITERATURE CITED.	52
BIOGRAPHICAL SKETCH	55

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1.1 Yields of tobacco, soybean and winter vetch planted in sequence in noninfested soil and soil infested with <u>Meloidogyne incognita</u> alone and <u>M. incognita</u> plus <u>Bacillus penetrans</u>	13
1.2 <u>Meloidogyne incognita</u> in soil and roots 0, 59 and 91 days after planting tobacco.	15
1.3 <u>Meloidogyne incognita</u> in soil 0, 72 and 107 days after planting soybean	16
1.4 <u>Meloidogyne incognita</u> in soil and roots 71 and 103 days after planting winter vetch	17
2.1 Yields of tobacco and soybean planted in sequence in noninfested soil and soil infested with <u>Meloidogyne incognita</u> alone and <u>M. incognita</u> plus <u>Bacillus penetrans</u> . . .	28
2.2 <u>Meloidogyne incognita</u> in soil and roots 0, 60 and 77 days after planting tobacco.	30
2.3 <u>Meloidogyne incognita</u> in soil and roots 39 and 118 days after planting soybean	31
3.1 The effect of number of juveniles and number of juveniles with spores of <u>Bacillus penetrans</u> on root penetration by <u>Meloidogyne incognita</u>	38
3.2 The effect of number of juveniles and number of juveniles with spores of <u>Bacillus penetrans</u> on the movement of <u>Meloidogyne incognita</u>	39
3.3 Number of galls on tomato roots 7 days after inoculation with 50 juveniles of <u>Meloidogyne incognita</u> noninfected (control) and infected with <u>Bacillus penetrans</u>	42

TablePage

3.4	Numbers of <u>Meloidogyne incognita</u> in greenhouse tomato roots 14 or 30 days after inoculation with juveniles not infected (control) and infected with <u>Bacillus penetrans</u>	44
3.5	Proportions of juveniles of <u>Meloidogyne incognita</u> not infected (control) and infected with <u>Bacillus penetrans</u> that moved more than 5.25 cm toward a tomato plant	45

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
2.1	Dosage-response function for inoculum of <u>Bacillus penetrans</u> added to field plots on 26 March 1983.	26
3.1	Spores of <u>Bacillus penetrans</u> attached to the cuticle of a second-stage juvenile of <u>Meloidogyne incognita</u>	41

Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

PATHOGENICITY OF Bacillus penetrans
TO Meloidogyne incognita

By

STEPHEN MICHAEL BROWN

April 1983

Chairman: G. C. Smart, Jr.

Major Department: Entomology and Nematology

The pathogenicity of Bacillus penetrans to Meloidogyne incognita was tested in field plots bordered by concrete, and the mode of action of B. penetrans on the nematode was investigated under controlled conditions. Bacillus penetrans was added to the soil of plots into which tobacco, soybeans, winter vetch, tobacco and soybeans were planted in sequence during the 1981 and 1982 growing seasons. Tobacco (1981 and 1982), winter vetch and soybeans (1982) showed trends of increasing yields with decreasing pathogenicity of M. incognita. There was insufficient evidence to declare a similar trend for soybeans during 1981. Yields from plots infested with M. incognita and treated with B. penetrans were 23% greater for tobacco (1981), 38% greater for winter vetch, 24% greater for tobacco (1982) and 35% greater for soybeans (1982) than those from plots infested with M. incognita alone.

In a laboratory experiment, juveniles of M. incognita infected with B. penetrans caused fewer galls (a total of 10) to form on the roots of tomato plants than did noninfected juveniles (a total of 61). Bacillus penetrans reduced the number of nematodes in tomato roots in two greenhouse experiments, and in a third experiment there was insufficient evidence to declare a lower number of nematodes in tomato roots inoculated with juveniles infected with B. penetrans than in roots inoculated with noninfected juveniles. Bacillus penetrans reduced the percentage of juveniles moving toward tomato plants in only one of four laboratory experiments.

Spores from the population of B. penetrans used in this study attached to M. javanica, M. incognita and M. arenaria. A greater proportion of juveniles of M. javanica were infected than those of either M. incognita or M. arenaria, and the corresponding proportions for M. incognita and M. arenaria were not found to be different.

INTRODUCTION

Cobb (1906) was the first to report distinctively shaped spores parasitizing nematodes. Numerous reports of nematodes with this parasite occurred thereafter. It is probable that all of these reported parasites are the same organism or closely related organisms. However, none of the authors who assigned a taxonomic position or proposed a life cycle for the organism obtained it in pure culture.

Thorne (1940) observed spores within the pseudocoelom and attached to the cuticle of a lesion nematode, Pratylenchus pratensis (de Man 1880) Filipjev 1936. On the basis of that observation only, he named the organism Duboscqia penetrans Thorne (Microsporidia, Nosematidae).

After observing smears and sections of parasitized nematodes with an electron microscope, Mankau (1975) renamed the organism Bacillus penetrans (Thorne) Mankau. He based the name change on an absence of both organelles and a membrane-surrounded nucleus, the presence of a typical bacterial cell wall and plasma membrane, and the presence of spores morphologically identical to spores of typical Bacillus spp. although shaped differently. The spores were refractive, a characteristic shared by the milky disease organisms B. popilliae and B. lentimorbus. Mankau (1975) also considered the disease in nematodes caused by B. penetrans as identical etiologically to the milky disease of scarabaeid grubs caused by B. popilliae and B. lentimorbus.

Unfortunately, none of the evidence given by Mankau (1975) is diagnostic for the genus Bacillus. No members of the family Bacillaceae produce

filamentous vegetative cells, and although the cells examined were prokaryote, they were not rod-shaped or spherical, and thus do not fit in Bacillaceae as presently defined. Bacterial cell walls and plasma membranes cannot be distinguished morphologically from corresponding eukaryotic structures. Prokaryotic cell walls are chemically distinctive from those of any eukaryotic cell, but Mankau (1975) did not report a chemical analysis of the cell wall of this organism. Moreover, a reliable chemical analysis can be carried out only from a pure culture, which has not been achieved for this organism.

Refractive endospores are not unique to Bacillus spp. Refractiveness is a characteristic of all endospores, which are defined as spores formed endogenously within a sporangium possessing a multilayered coat, a cortex and a contained cytoplasm or core that is capable of resisting high temperatures and exposure to radiation (Cross 1970). In addition to Bacillaceae, endospores have been shown to be formed by some Actinomycetes (Cross et al. 1968, Dorokhova et al. 1968).

The similarities between the nematode disease and the milky disease of insects lie only in that the pathogens are bacteria and that multiplication occurs in a clear body fluid. Spores of B. penetrans attach to the cuticle of nematodes when nematodes contact them in soil. After attachment, a spore germinates and a germ tube penetrates the body wall (Allen 1957, Mankau et al. 1976, Sayre and Wergin 1977). Insect larvae ingest B. popilliae spores that then germinate in the midgut, producing motile rods. Splittstoesser et al. (1975) proposed that the motile rods penetrate the midgut wall, although vegetative cell contact with the midgut epithelium has not been observed (Splittstoesser et al. 1978). They suggested that penetration may occur by phagocytosis of the midgut cells similar to the

invasion of honey bee larvae by B. larvae (Davidson 1973). Splittstoesser et al. (1978) and Kawanishi et al. (1978) documented the interaction of insect midgut cells and B. popilliae, although penetration of the basement membrane and entry into the hemocoel was not observed.

Sayre and Wergin (1977) suggested that B. penetrans might be included among genera of the order Actinomycetales, Actinomycetes--Part 17 of Bergey's Manual (1974). They studied parasitized specimens of a root-knot nematode, Meloidogyne incognita (Kofoed and White 1919) Chitwood 1949, (smears and sections of adult females and whole juveniles), but again the parasite was not studied from a pure culture. They gave three reasons for a relationship to Actinomycetales: an absence of both organelles and a membrane-surrounded nucleus; the presence of branching filamentous mycelia, which are less than 1 μm in diameter; and a double-track cell wall with hairlike projections similar to that of several species of Actinomyces described by Slack and Gerencser (1975). Sayre and Wergin (1977) concluded that a precise classification could not be made until the organism had been cultivated in vitro. They called the organism a bacterial spore parasite of nematodes (BSPN).

Although the absence of organelles and of a membrane-surrounded nucleus and the presence of vegetative cells with narrow branching mycelia suggest a bacterium related to Actinomycetes, a double-track cell wall with fimbriae is not diagnostic for Actinomycetales (Bergey's Manual 1974). No existing evidence precludes B. penetrans from the order Actinomycetales. Although the classification of this organism is in question, it will be called Bacillus penetrans in this study.

The experimental evidence that supports the pathogenicity of B. penetrans to nematodes has been derived from greenhouse and laboratory studies.

Prasad (1971) reported that infected juveniles of M. javanica (Treub 1885) Chitwood 1949 produced fewer galls on tomato roots (Lycopersicon esculentum Miller) than did noninfected juveniles under greenhouse conditions. Tomato plants inoculated with M. incognita had more leaves and fewer root galls when grown under greenhouse conditions in soil containing B. penetrans than when grown in steamed soil without B. penetrans (Prasad 1971). Greenhouse snap beans (Phaseolus vulgaris L.) inoculated with Pratylenchus scribneri Steiner 1943 had fewer nematodes after 55 days when grown in soil containing B. penetrans than when grown in soil without B. penetrans. The total number of P. scribneri collected from soil and roots was 1220 from the treatment with B. penetrans compared with 3034 from the treatment without B. penetrans. However, the population size of P. scribneri from soil containing B. penetrans may have been underestimated, since the Baermann funnel technique was used to extract the nematodes from the soil and roots. Using a modified Baermann funnel technique (a petri plate replaced the funnel), Prasad (1971) demonstrated that the number of M. javanica extracted from soil containing B. penetrans was less than that from steamed soil. He collected 279 of 2000 M. javanica juveniles from soil containing B. penetrans compared with 1719 of 2000 from steamed soil. If B. penetrans has a similar effect on P. scribneri, the population size of P. scribneri in soil infested with B. penetrans may have been underestimated.

Worldwide, the root-knot nematodes, Meloidogyne spp., are the most economically important group of plant-parasitic nematodes (Franklin 1979, Lamberti 1979, Sasser 1979). They are geographically widespread, have a wide host range and cause crop damage both alone and together with other plant pathogens (Sasser 1980). Although the genus contains more than 40

species, M. incognita is the most important species throughout the world (Sasser 1980).

If the pathogenicity of M. incognita to plants could be hindered by B. penetrans over several growing seasons, B. penetrans might be suitable to include as a component of a biological control program where a combination of pathogens, resistant varieties and crop rotations are used to reduce damage to crops caused by M. incognita.

The purpose of this study was to determine the practicality of using B. penetrans against M. incognita under field conditions and to determine aspects of the mode of action of B. penetrans. In addition, M. javanica and M. arenaria (Neal 1889) Chitwood 1949 as well as M. incognita were exposed to B. penetrans to determine if the three species would become infected.

CHAPTER 1
INCREASED CROP YIELDS FOLLOWING APPLICATION OF
Bacillus penetrans TO PLOTS INFESTED
WITH Meloidogyne incognita

Introduction

Prasad (1971) reported that greenhouse tomatoes inoculated with Meloidogyne incognita (Kofoed and White) Chitwood had improved growth and that greenhouse snap beans had fewer nematodes 55 days after inoculation with Pratylenchus scribneri Steiner when grown in soil containing B. penetrans than when grown in soil without the antagonist. Should this bacterium prove to be pathogenic to plant-parasitic nematodes under field conditions, damage to plants could be reduced by applying spores of B. penetrans to soil.

Several advantages of this bacterium for control of nematodes are known. Bacterial spores are resistant to heat, desiccation and many chemicals. Although information about predation is scant (Alexander 1977), spores may have few antagonists. Four populations of B. penetrans have shown host specificity (Prasad 1971, Dutky 1978). Therefore, it may be possible to select isolates pathogenic to plant parasites leaving saprophagous and predatory nematodes unaffected.

Disadvantages that may limit the use of this bacterium are that its spores are nonmotile and apparently attach to the cuticle of nematodes only when nematodes contact them in soil. Of most importance, B. penetrans has not been grown in vitro. The fact that it now can be grown only in a

nematode host hinders experimentation and may prevent commercial production of inoculum.

The purpose of this chapter was to determine if the addition of spores of B. penetrans to soil hindered the parasitism of M. incognita to three crops planted in sequence.

Materials and Methods

In the first year of this experiment, a population of M. incognita was established in eight of 12 plots located in Gainesville, Florida, while a population of B. penetrans was produced in a greenhouse. The following year B. penetrans was added to four of the plots infested with M. incognita and three crops were planted in sequence: tobacco (Nicotiana tabacum L. 'NC 2326'), soybeans (Glycine max (L.) Merrill 'Hood') and winter vetch (Vicia villosa Roth).

In spring 1980, twelve 2.4-m x 0.9-m concrete-bordered plots containing Arredondo fine sand (92.9% sand, 4.9% silt and 2.2% clay) to a depth of 60 cm were treated with methyl bromide (53 g/m^2) under plastic. One month after fumigation, okra (Hibiscus esculentus L. 'Clemson Spineless') was planted in all plots. The plots were grouped into four blocks, and three treatments were assigned randomly within the blocks in a randomized, complete-block design. The treatments were 1. M. incognita alone, 2. M. incognita plus B. penetrans and 3. neither M. incognita nor B. penetrans. Forty-five days after planting okra, the soil of treatments 1 and 2 were infested with eggs of M. incognita (300,000/plot). The eggs were obtained by shaking tomato roots (cultivar Rutgers) infected with M. incognita in a 0.5% sodium hypochlorite solution for 30 sec (Hussey and Barker 1973).

In late fall, okra was removed from the plots and sweet blue lupine (Lupinus angustifolius L. 'Frost') was planted. In early spring 1981, the lupine was turned into the soil. Okra and lupine were grown to distribute the population of M. incognita as might be found in agricultural soil.

Concurrent with the establishing of M. incognita in the field plots, B. penetrans was produced in M. incognita. Juveniles of Meloidogyne spp. parasitized by B. penetrans were found in association with Japanese privet (Ligustrum japonicum Thunberg) from MacClenny, Florida. The isolated juveniles were suspended in water and injected into the soil of potted greenhouse tomato plants (cultivar Rutgers) that then were grown for approximately three months. The tomato roots either were cut into pieces and mixed with the soil or comminuted in a blender, filtered through a coarse screen and the filtrate poured into the soil. In both cases the soil was air-dried for one month. The dried soil was diluted (1 to 1) with fumigated potting soil (90.6% sand, 3.9% silt and 5.5% clay), planted with tomato seedlings and reinfested with healthy juveniles of M. incognita.

From spring 1981 to spring 1982, three crops (tobacco, soybean, winter vetch) were planted in sequence to test the hypotheses that the yields of the crops increase as the pathogenicity of M. incognita decreases. With the model for a randomized, complete-block design, $X_{ij} = \mu + \beta_i + \tau_j + \epsilon_{ij}$, where μ represents a location parameter, β_i represents the effect of the i^{th} block and τ_j represents the effect of the j^{th} treatment and is subject to the constraint $\sum_j \tau_j = 0$; the error terms (ϵ_{ij} 's) are mutually independent random variables from some continuous population that is symmetric about zero; the null hypothesis of no treatment effect, $H_0: \tau_1 = \tau_2 = \tau_3$, was tested against the ordered alternative research hypothesis, $H_1: \tau_1 \leq \tau_2 \leq \tau_3$, where at least one of these inequalities is strict. Here, τ_1 is

the effect of the presence of nematodes on yield, τ_2 is the effect of the presence of both nematodes and bacteria on yield, and τ_3 is the effect of having no nematodes or bacteria on yield. Because the assumption of normally distributed error terms seemed questionable, a nonparametric test specifically designed to detect these ordered alternatives was used. The composite Wilcoxon test, proposed by Kepner and Robinson (1982) was selected for use in this research. A significant omnibus test for ordered alternatives was followed with point estimates of the differences between treatment effects based on one-sample median estimators (Hollander and Wolfe 1973). Had the omnibus test not been significant, estimates of treatment effects would not have been made. It was decided that pairwise comparisons to determine which treatment effects were strictly ordered should not be conducted because of the small sample sizes.

Samples of nematodes from soil and roots (except for soybeans) were taken during the growing of the three crops. The effect on the nematode population of adding B. penetrans to soil was tested with the Wilcoxon signed rank test (Hollander and Wolfe 1973). The proportions of parasitized nematodes in the samples taken at harvest were compared by normal approximation. Also biological assays on the soil samples at harvests were carried out to provide additional estimates of the proportion of parasitized nematodes in the population. The independence of the proportion of parasitized juveniles and crop type was investigated with the log likelihood ratio G statistic of Sokal and Rohlf (1981).

On 10 April 1981 soil samples were taken from all plots to determine the nematode densities, B. penetrans was added to the soil of treatment 2 and tobacco was planted in all plots. Six soil cores (2.5-cm diam x 25-cm deep) were collected randomly 15 cm from the planting sites of tobacco plants.

The soil was mixed and nematodes were extracted from the soil by a procedure adapted from Jenkins (1964). A 250-cm³, random subsample of soil was rinsed into a container and mixed with a stream of water to a volume of approximately 500 ml. One minute later the water was decanted onto stacked sieves of 75- μ m and 25- μ m openings (200 and 500 mesh). Mixing and decanting were repeated three times. The residue from the 25- μ m sieve was washed into a 50-ml centrifuge tube and spun in a centrifuge for 5 min at a relative centrifugal force of 1071 *g*. The supernatant was discarded, and the pellet was mixed in a sugar solution (454 g/l) and spun for 2 min at the same force. The supernatant was decanted onto a 25- μ m sieve, and the residue from the sieve was collected and diluted to 10 ml. The nematodes from a 1-ml sample were counted in a Peters counting slide. When nematodes were too numerous to count, a greater dilution was used, and the number of nematodes was converted to nematodes per 25 cm³ of soil. Bacillus penetrans was added to the soil of treatment 2 by placing approximately 1200 cm³ of potting soil infested with B. penetrans into six 10-cm diam x 15-cm deep holes into which tobacco was transplanted. Fumigated potting soil was placed similarly into the soil of the other two treatments, and six tobacco seedlings were transplanted into each of those plots.

During the growing of tobacco, samples of nematodes in soil and roots were taken at 59 and 91 days (final harvest) after transplanting. The nematodes in soil were estimated as described previously, and roots from the randomly selected 250-cm³ subsamples of soil were stained with acid fuchsin in lactophenol to aid in counting the nematodes (Southey 1970). After the nematodes were counted, the roots were oven-dried, and the number of nematodes was converted to nematodes per gram of dry roots. Tobacco leaves were harvested six times at 7-day intervals beginning 56 days after transplanting. Leaves were dried at 65 C for 7 days before weighing.

Biological assays of soil were taken at the harvest of each crop to determine whether the proportions of parasitized juveniles followed the same trend as those obtained from field sampling. A 500-cm³, random sample of soil taken from the soil collected at harvest from each plot of the treatment with nematodes and bacteria was air-dried for approximately 2 months in the laboratory. Three 30-cm³, random subsamples of dried soil from each plot were placed in 30-ml plastic cups, moistened with 10 ml of distilled water and incubated for 3 days at 28 C. Fumigated potting soil was used as a control. Then 1000 juveniles of M. incognita in water were injected into each cup and incubated for 3 days at 28 C. The juveniles were extracted from the soil by the method described previously, and a random sample of 50 juveniles was examined (at 200X) for parasitism by B. penetrans. Moistening the soil for 3 days before adding nematodes was selected following a previous experiment comparing moistening the soil for 3 days with no prior moistening before adding nematodes to soil. The proportions of parasitized nematodes from these treatments were compared by normal approximation.

After the last tobacco harvest, the control plots were treated with methyl bromide (210 g/m²) to kill the contaminating nematodes. On 27 July 1981, 11 days after the application of methyl bromide, soybeans were planted in the plots in two 2.4-m rows spaced 40 cm apart. Seven days later the seedlings were thinned to a 5-cm to 7.5-cm spacing. Soil samples were taken and processed for nematodes at 0, 72 and 107 days (harvest) after planting. The number of soil cores was increased to 15 at the 107-day sample. After harvest and before weighing, seeds were allowed to reach a mean moisture content of 9.46% (s = 0.19, with all observations $\pm$ 2s of the mean).

On 9 December 1981, 28 days after the soybeans had been harvested, winter vetch was planted. The final sample of nematodes in soil from soybeans was used as a preplant sample for winter vetch. Soil samples from winter vetch were collected randomly 71 and 103 days (harvest) after planting. At harvest, roots from a random sample of five plants from each plot were collected and cut into small pieces. A 2-g subsample of roots was selected randomly and stained with acid fuchsin in lactophenol to aid in counting nematodes. The winter vetch forage was dried at 65 C for 7 days before weighing.

Results

The test of Kepner and Robinson (1982) indicated a trend of increasing yields with decreasing pathogenicity of the M. incognita population for both tobacco and winter vetch (Table 1.1). The attained significance level of the tests, which is the probability of observing a value of the statistic at least as extreme as the value observed, was $P = .012$ for tobacco and $P = .008$ for winter vetch. The same test provided insufficient evidence to enable one to conclude a trend of increasing yield with decreasing pathogenicity of the nematode population for soybeans ($P = .23$). The dry leaf weight of tobacco (based on one sample median estimators) from plots infested with nematodes and bacteria minus that from plots infested with nematodes alone was 150 g, and between the control minus the treatment with nematodes alone was 653 g. The increase in yield for plots infested with nematodes and bacteria over those infested with nematodes alone was 23% of the increase in yield observed for the control plots over the plots infested with nematodes alone. The dry forage weight of winter vetch from plots infested with nematodes and bacteria minus that from plots infested with nematodes alone was 122 g, and between the control minus the treatment with

Table 1.1 - Yields of tobacco, soybean and winter vetch planted in sequence in noninfested soil and soil infested with Meloidogyne incognita alone and M. incognita plus Bacillus penetrans.

Treatment	Weight (g)			
	Tobacco leaf (dry)		Soybean seed	
Control	976	1076	1253	965*
			808	619
			606	732
Nematodes + Bacteria	592	460	1011	334
			784	760
			729	772
Nematodes	375	618	363	256
			512	495
			668	809
			736	632
			792	580
			373	426
			576	805
			227	311
			484	440

*Values are from four blocks of a randomized, complete-block design.

nematodes alone was 324 g. The increase in yield for plots infested with nematodes and bacteria over those infested with nematodes alone was 38% of the increase in yield observed for the control plots over the plots infested with nematodes alone.

The Wilcoxon signed rank test indicated fewer nematodes in the plots with nematodes and bacteria than in the plots with nematodes alone at the 59-day sample of tobacco soil ($P = .062$), and more nematodes in the plots with nematodes and bacteria than in the plots with nematodes alone at the 91-day sample of soil and tobacco roots ($P = .062$) and at the 0-day sample of soybean soil ($P = .062$) (Tables 1.2 and 1.3). In the remaining samples of soil and roots, there was insufficient evidence to enable one to declare that the numbers of nematodes in the plots with nematodes and bacteria and with nematodes alone were different (Tables 1.2, 1.3, and 1.4).

The proportions of parasitized juveniles of M. incognita in the final soil samples of each crop were tobacco, 40 of 1192 (3.4%); soybeans, 41 of 951 (4.3%); and winter vetch, 39 of 381 (10.2%). Winter vetch had a greater proportion of nematodes with spores on the cuticle than either tobacco or soybean, and the corresponding proportions for tobacco and soybean were not found to be different. The significance level for this family of comparisons (the probability of making at least one Type I error in the three conclusions) was $\alpha = .03$.

The proportions of parasitized juveniles of M. incognita in biological assays of the soil collected at harvests were tobacco, 168 of 600; soybeans 155 of 600; and winter vetch, 358 of 600. The hypothesis that the true proportions of parasitized nematodes in the soil from the three crops are simultaneously equal was rejected ($P < .001$) by the log likelihood ratio test. Follow-up pairwise comparisons among the proportions indicated

Table 1.2 - *Meloidogyne incognita* in soil and roots 0, 59 and 91 days after planting tobacco.

Days After Planting	Block	Treatment		
		Control	Nematodes + Bacteria	Nematodes
0	1	(0,-)*	(12,-)	(6,-)
	2	(0,-)	(12,-)	(4,-)
	3	(0,-)	(5,-)	(8,-)
	4	(0,-)	(21,-)	(6,-)

59	1	(0,63)	(1540,1579)	(1935,1659)
	2	(0,0)	(790,3000)	(1090,1567)
	3	(0,11)	(425,2538)	(1290,908)
	4	(0,179)	(730,5786)	(890,2025)

91	1	(288,500)	(2930,3067)	(1445,2234)
	2	(2,13)	(4370,2700)	(1895,1645)
	3	(190,181)	(2400,734)	(1085,0)**
	4	(1680,338)	(2220,8300)	(1885,0)**

* In the ordered pair (A,B), A is the number of juveniles/25 cm³ of soil, and B is the number of nematodes/g of dry roots. A dash (-) for B indicates that samples were not taken.

** No roots were present.

Table 1.3 - Meloidogyne incognita in soil 0, 72 and 107 days after planting soybean.

<u>Days After Planting</u>	<u>Block</u>	<u>Treatment</u>		
		<u>Control</u>	<u>Nematodes + Bacteria</u>	<u>Nematodes</u>
0	1	0*	375	135
	2	0	1175	715
	3	0	185	135
	4	0	305	295

72	1	0	419	174
	2	0	197	345
	3	0	105	347
	4	0	146	161

107	1	0	338	248
	2	0	190	235
	3	0	248	916
	4	0	175	342

* Values are the number of juveniles/25 cm³ of soil.

Table 1.4 - Meloidogyne incognita in soil and roots 71 and 103 days after planting winter vetch.

Days After Planting	Block	Treatment		
		Control	Nematodes + Bacteria	Nematodes
71	1	(0,-)*	(70,-)	(49,-)
	2	(0,-)	(49,-)	(41,-)
	3	(0,-)	(52,-)	(174,-)
	4	(0,-)	(38,-)	(64,-)

103	1	(0,0)	(79,152)	(48,318)
	2	(0,0)	(98,357)	(61,171)
	3	(0,0)	(36,160)	(68,409)
	4	(0,0)	(168,108)	(64,280)

* In the ordered pair (A,B), A is the number of juveniles/25 cm³ of soil, and B is the number of nematodes/g of fresh roots. A dash (-) for B indicates that samples were not taken.

that the proportion of nematodes with spores on the cuticle from soil collected at harvest of winter vetch exceeded those from the preceding crops, tobacco and soybean ($P < .0001$ for each test). No difference could be claimed between the proportions for tobacco and soybean ($P = .1977$).

Spores were observed on 119 of 200 randomly selected M. incognita juveniles in a biological assay of the same potting soil used to infest the treatment with nematodes and bacteria before planting tobacco.

The proportions of infected nematodes from the experiment comparing bacteria-infested soil moistened for 0 or 3 days before adding test nematodes were 59 of 200 for 0 days and 146 of 200 for 3 days. The hypothesis that the proportions are equal was rejected by normal approximation ($P < .0001$).

Samples of mature females from roots collected at harvest of tobacco and winter vetch were examined for spores within the body. The proportions of parasitized females were tobacco, 8 of 42, and winter vetch, 0 of 40. The number of parasitized females from roots of winter vetch was lower than that from roots of tobacco ($P = .0008$).

Discussion

The increased yields of tobacco and winter vetch resulting from adding B. penetrans to field soil containing an established population of M. incognita demonstrated that B. penetrans reduced the pathogenicity of the population of M. incognita. Prasad (1971) reported that greenhouse tomatoes inoculated with M. incognita had more leaves when grown in soil containing B. penetrans than when grown in steamed soil. The effect of B. penetrans on M. incognita now can be extended to field conditions. Fifteen days after planting soybean seed, leaf chlorosis caused by

Rhizoctonia sp. was observed on soybean plants only in the control plots. Probably Rhizoctonia sp. was introduced into plots on soybean seed and thrived in the fumigated soil of the control. The effect of the disease on yield could not be determined, but the possibility of a suppressed yield for the control should be considered when interpreting the results.

Prasad (1971) reported that greenhouse tomatoes inoculated with M. incognita had fewer nematode galls on roots and that greenhouse snap beans inoculated with P. scribneri had fewer nematodes after 55 days when grown in soil containing B. penetrans. However, the population of P. scribneri from soil containing B. penetrans may have been underestimated, since the Baermann funnel technique was used to extract the nematodes from the soil and roots. Using a modified Baermann funnel technique (a petri plate replaced the funnel), Prasad (1971) demonstrated that the number of M. javanica (Treub) Chitwood extracted from soil containing B. penetrans was less than that from soil without the antagonist. He collected 279 of 2000 M. javanica juveniles from soil containing B. penetrans compared with 1719 of 2000 from steamed soil. If B. penetrans has a similar effect on P. scribneri, the population of P. scribneri in soil infested with B. penetrans may have been underestimated. I suspect that a low soil concentration of B. penetrans causes a reduction in the rate of population growth of M. incognita. If parasitized juveniles are prevented from feeding or if feeding is hampered, the rate of nematode population growth is decreased. The lower number of juveniles at the 59-day sample of tobacco soil in plots treated with bacteria may be a result of a reduced rate of population growth. The lower number of nematodes in soil and roots was a result of the reduced carrying capacity of the tobacco plants. In two of the four plots containing

nematodes only, all of the plants were dead at the end of the experiment (Table 1.2).

The proportion of M. incognita juveniles with spores on the cuticle was greater in the final soil samples from winter vetch than in those from tobacco or soybean. It is conjectured that the larger proportion of parasitized juveniles at the end of the cultivation of the three crops indicated that the number of B. penetrans in the soil increased. Alexander (1981) reviewed studies of the addition of bacteria to natural ecosystems that suggested that for bacteria that persist in soil there is a characteristic number that is maintained. Determining this characteristic number for B. penetrans in agricultural soil, and in turn, the optimum effect of that concentration of B. penetrans on a population of plant parasitic nematodes would be an interesting continuation of the present study. Such work would be aided if an artificial medium for growing B. penetrans were developed. In addition, this would lead to a pure culture of B. penetrans allowing the study of its biology and taxonomy.

Summary

The pathogenicity of Bacillus penetrans to Meloidogyne incognita was tested under field conditions. Bacillus penetrans was added to concrete-bordered plots into which tobacco, soybeans and winter vetch were planted in sequence. Tobacco and winter vetch showed trends of increasing yields with decreasing pathogenicity of M. incognita. There was insufficient evidence to declare a similar trend for soybeans. Yields from plots infested with M. incognita and treated with B. penetrans were 23% greater for tobacco and 38% greater for winter vetch than those from plots infested with M. incognita alone. Soil samples taken midway through

the tobacco season revealed fewer second-stage-juvenile nematodes in plots infested with B. penetrans than in the nematode-infested plots without B. penetrans. The proportion of juvenile nematodes infected with B. penetrans was greater at the harvest of winter vetch than at the harvests of tobacco or soybeans, while the proportion of mature females infected with B. penetrans was less at the harvest of winter vetch than at the harvest of tobacco.

CHAPTER 2
INCREASED CROP YIELDS FOLLOWING APPLICATION OF
Bacillus penetrans TO PLOTS INFESTED WITH
B. penetrans AND Meloidogyne incognita

Introduction

The purpose of this chapter was to determine whether adding inoculum of Bacillus penetrans to plots infested during the previous year with Meloidogyne incognita and B. penetrans would decrease further the effects of M. incognita to two crops planted in sequence. In addition, M. javanica and M. arenaria as well as M. incognita were exposed to B. penetrans to determine if the three species would become infected.

Materials and Methods

Field Experiments

Tobacco (Nicotiana tabacum L. 'NC 2326') and then soybeans (Glycine max (L.) Merrill 'Hood') were grown during the 1982 growing season in twelve 2.4-m x 0.9-m concrete-bordered plots containing Arredondo fine sand (92.8% sand, 4.9% silt and 2.2% clay) to a depth of 60 cm in which three crops had been grown in sequence from spring 1981 to spring 1982 (CHAPTER 1). The plots were assigned randomly to the 12 block-by-treatment combinations in a randomized, complete-block design involving four blocks and three treatments. The treatments were 1. M. incognita alone, 2. M. incognita plus B. penetrans

and 3. neither M. incognita nor B. penetrans. The procedures used to infest the plots initially with these organisms are described in CHAPTER 1. In spring 1982 additional inoculum of B. penetrans was added to the plots containing M. incognita and B. penetrans. This inoculum was produced by air-drying and grinding tomato root systems previously colonized by M. incognita parasitized by B. penetrans (Stirling and Wachtel 1980). The original source of B. penetrans was MacClenny, Florida.

Tobacco and then soybeans were planted in spring and summer 1982 to test the hypotheses that the yields of the two crops increase as the pathogenicity of M. incognita decreases. The composite Wilcoxon test (Kepner and Robinson 1982) was used to test the null hypothesis of no treatment effect, $H_0: \tau_1 = \tau_2 = \tau_3$, against the ordered alternative research hypothesis, $H_1: \tau_1 \leq \tau_2 \leq \tau_3$, where at least one of the inequalities is strict. Here, τ_1 is the effect of the presence of nematodes on yield, τ_2 is the effect of the presence of both nematodes and bacteria on yield, and τ_3 is the effect of having no nematodes or bacteria on yield. A significant omnibus test for ordered alternatives was followed with point estimates of the differences between treatment effects based on one-sample median estimators (Hollander and Wolfe 1973). If the omnibus test was not significant, no point estimates were to be made. Samples of nematodes from soil and roots were taken during the growing of the two crops. The effect on the nematode population of adding B. penetrans to soil was analyzed with the Wilcoxon signed rank test (Hollander and Wolfe 1973). The proportions of infected nematodes were compared by normal approximation.

On 26 March 1982, 150 g of inoculum of B. penetrans were incorporated into the top 2.5 cm of soil in the plots containing M. incognita and B. penetrans. Soil samples were taken from all plots to determine the

densities of nematodes (CHAPTER 1), and six tobacco seedlings were transplanted into each plot. During the growing of tobacco, samples of nematodes in soil were taken 60 and 77 days (final harvest) after transplanting, and samples of nematodes in roots were taken 77 days after transplanting. The nematodes in soil were estimated from 15 soil cores (2.5-cm diam x 25-cm deep) collected randomly 15 cm from the planting sites of tobacco. The cores were mixed, and the nematodes were extracted as described previously (CHAPTER 1) from a random subsample of 250 cm^3 of soil. The nematodes in roots were estimated from a random sample of 1 g of roots/plot. The samples of roots were stained with acid fuchsin in lactophenol to aid in counting the nematodes (Southey 1970). Tobacco leaves were harvested four times at 7-day intervals beginning 56 days after transplanting. Leaves were dried at 65 C for 7 days before weighing.

After the last tobacco harvest, the control plots were treated with methyl bromide (210 g/m^2) under plastic to kill the contaminating nematodes. On 23 June 1982, 12 days after the final harvest of tobacco, soybeans were planted in the plots in two 2.4-m rows spaced 40 cm apart. Seven days later the seedlings were thinned to a 5-cm to 7.5-cm spacing. The final sample of nematodes in soil for tobacco was used as a preplant sample for soybeans. Soil samples were processed for nematodes at 39 and 118 days (harvest) after planting. The nematodes in roots were estimated, as described for tobacco, 39 days after planting. Soybean seed weight was reported at 11% seed moisture.

Determination of a Dosage-Response Function for Inoculum of *Bacillus penetrans*

Biological assays of the inoculum were conducted to determine the percentage of juveniles of *M. incognita* infected when varying amounts of

inoculum were used. Zero, 10, 100, and 1000 mg of inoculum were added to 30-ml plastic cups and mixed with 30 g of quartz sand; each amount of inoculum was replicated five times. The mixture of inoculum and sand was moistened with 10 ml of distilled water and incubated for 3 days at 28 C. Then 1000 juveniles of M. incognita in water were injected into each cup and incubated for 3 days at 28 C. The juveniles were extracted from the cups by modifications (CHAPTER 1) of the sieving and centrifugation procedure of Jenkins (1964), and a random sample of 50 juveniles was examined (at 200X) for attached spores of B. penetrans.

A third-order-polynomial regression model was fit to this data using the Statistical Analysis System (SAS) to express the percentage of infected nematodes as a cubic function of the base 10 log of 1 mg more than the weight (in mg) of the inoculum (Fig. 2.1). A residual analysis then was performed to determine which, if any, of the model assumptions failed to be satisfied (Neter and Wasserman 1974). The observation $y = 14$ at $\log(100 + 1) = 2$ was determined to be an outlier by fitting a cubic regression to the remaining $n-1$ observations and constructing a 95% prediction interval for a new random response at $\log(100 + 1) = 2$. Because the response $y = 14$ fell outside of this prediction interval, it was considered as not having come from the same population as the other $n-1$ observations and was deleted from further study.

Attachment of Spores to Meloidogyne javanica, M. incognita and M. arenaria

Biological assays were used to determine the relative attachment of spores to three species of Meloidogyne: M. javanica, M. incognita and M. arenaria. Potting soil (90.6% sand, 3.9% silt and 5.5% clay) infested with B. penetrans was used in the assays using the plastic-cup procedure

Figure 2.1 - Dosage-response function for inoculum of Bacillus penetrans added to field plots on 26 March 1983. The observation $y = 14$ was considered an outlier ($P < .05$).

previously described with four replications for each species. The independence of the percentage of infected juveniles and species was investigated with the log likelihood ratio G statistic of Sokal and Rohlf (1981).

Results

Field Experiments

The test of Kepner and Robinson (1982) indicated a trend of increasing yields with decreasing pathogenicity of M. incognita for both tobacco and soybean (Table 2.1). The attained significance level of the tests was $P = .004$ for tobacco and $P = .008$ for soybeans (the attained significance level of a test is the probability of observing a value of the statistic at least as extreme as the value observed). The dry leaf weight of tobacco from plots infested with nematodes and bacteria minus that from plots infested with nematodes alone was 235 g, and between the control minus the treatment with nematodes alone was 962 g. The increase in yield for plots infested with nematodes and bacteria over those infested with nematodes alone was 24% of the increase in yield observed for the control plots over the plots infested with nematodes alone. The seed weight of soybeans from plots infested with nematodes and bacteria minus that from plots infested with nematodes alone was 149 g, and between the control minus the treatment with nematodes alone was 431 g. The increase in yield for plots infested with nematodes and bacteria over those infested with nematodes alone was 35% of the increase in yield observed for the control plots over the plots infested with nematodes alone.

The Wilcoxon signed rank test indicated fewer nematodes in the plots with nematodes and bacteria than in the plots with nematodes alone at the

Table 2.1 - Yields of tobacco and soybean planted in sequence in non-infested soil and soil infested with Meloidogyne incognita alone and M. incognita plus Bacillus penetrans.

Treatment	Weight (g)							
	Tobacco leaf (dry)				Soybean seed			
Control	968	1000	1062	1076*	1475	1365	1301	1412
Nematodes + Bacteria	733	379	273	102	1165	1060	1194	1043
Nematodes	60	228	0	0	934	942	913	1107

* Values are from four blocks of a randomized, complete-block design.

118-day (harvest) sample of soybean soil ($P = .062$). In the remaining samples of soil and roots, there was insufficient evidence to enable one to declare that the numbers of nematodes in the plots with nematodes and bacteria and with nematodes alone were different (Tables 2.2 and 2.3).

The proportion of infected juveniles in the soil sample taken from the treatment with M. incognita plus B. penetrans before planting tobacco was 39 of 381 (10.2%). The proportions of infected juveniles in the final soil samples of each crop were tobacco, 309 of 1647 (18.8%) and soybeans, 192 of 901 (21.3%). Soybeans had a greater proportion of infected juveniles than that of tobacco ($P = .063$). Samples of mature females from roots collected at harvest of tobacco and 39 days after planting soybeans were examined (at 400X) for spores within the body. The proportions of females with spores within the body were tobacco, 8 of 40, and soybeans, 3 of 40. The proportion of females containing spores from roots of soybeans was lower than that of tobacco ($P = .0495$).

Determination of a Dosage-Response Function for the Inoculum

A third-order-polynomial regression function for the percentage of infected juveniles (y) versus the log of 1 mg more than the weight (in mg) of the inoculum (x) is $y = -49x + 91x^2 - 24x^3$ (Fig. 2.1).

Attachment of Spores to Meloidogyne javanica, M. incognita and M. arenaria

Spores of population of B. penetrans attached to the cuticle of all three nematode species. The proportions of infected juveniles of the three species of Meloidogyne were M. javanica, 157 of 200; M. incognita, 95 of 200; and M. arenaria, 105 of 200. A greater proportion of juveniles of M. javanica were infected than were those of either M. incognita or M. arenaria, and the corresponding proportions for M. incognita and M. arenaria were not

Table 2.2 - *Meloidogyne incognita* in soil and roots 0, 60 and 77 days after planting tobacco.

Days After Planting	Block	Treatment		
		Control	Nematodes + Bacteria	Nematodes
0	1	(0,-)*	(79,-)	(48,-)
	2	(0,-)	(98,-)	(61,-)
	3	(0,-)	(36,-)	(68,-)
	4	(0,-)	(168,-)	(64,-)

60	1	(63,-)	(16,-)	(69,-)
	2	(1,-)	(95,-)	(314,-)
	3	(0,-)	(94,-)	(155,-)
	4	(0,-)	(207,-)	(143,-)

77	1	(252,63)	(881,115)	(167,163)
	2	(0,2)	(203,228)	(389,92)
	3	(12,5)	(358,172)	(57,0)**
	4	(0,0)	(205,254)	(25,0)**

* In the ordered pair (A,B), A is the number of juveniles/25 cm³ of soil, and B is the number of nematodes/g of fresh roots. A dash (-) for B indicates that samples were not taken.

** No roots were present.

Table 2.3 - *Meloidogyne incognita* in soil and roots 39 and 118 days after planting soybean.

Days After Planting	Block	Treatment		
		Control	Nematodes + Bacteria	Nematodes
39	1	(0,0)*	(55,196)	(13,241)
	2	(0,0)	(54,97)	(33,136)
	3	(0,0)	(9,55)	(11,143)
	4	(0,0)	(20,134)	(7,80)

118	1	(0,-)	(142,-)	(271,-)
	2	(0,-)	(254,-)	(371,-)
	3	(0,-)	(208,-)	(786,-)
	4	(0,-)	(297,-)	(330,-)

* In the ordered pair (A,B), A is the number of juveniles/25 cm³ of soil, and B is the number of nematodes/g of fresh roots. A dash (-) for B indicates that samples were not taken.

found to be different. The significance level for this family of comparisons (the probability of making at least one Type I error in the four conclusions) was $\alpha = .04$. Nearly all infected M. javanica and the majority of infected M. incognita had more than 15 attached spores; the infected M. arenaria had fewer than seven attached spores, except for one specimen that was heavily infected.

Discussion

The increased yields of tobacco and soybeans from field plots infested with B. penetrans and M. incognita over those infested with M. incognita alone agree with previous field experiments (CHAPTER 1) and provide further supporting evidence that B. penetrans is pathogenic to M. incognita under field conditions. The addition of B. penetrans at the beginning of this experiment may have contributed to the increased incidence of infection of juveniles in soil; however, this incidence of infection did not affect yield appreciably when compared with those reported previously (CHAPTER 1).

A tentative conclusion that B. penetrans reduces the population size of M. incognita could be drawn from the samples of juveniles in soil at the harvest of soybeans. However, the size of the population at a given time is dependent on so many factors that a generalization is difficult to make. For example, a high initial infestation of juveniles may damage a host to such a degree that subsequent lack of food limits the increase in nematode numbers. On the other hand, if juveniles are in some way inhibited from penetrating roots or developing once within the roots, damage to the host may be less and juveniles that do successfully complete

their life cycles may produce more offspring due to less competition. As a result of this early protection of the host, the size of the population may increase later in the season. Future research should emphasize studies on the mode of action of B. penetrans to root-knot nematodes.

This population of B. penetrans attaches to M. javanica and M. arenaria as well as M. incognita. Assuming that attachment of spores indicates a host-pathogen relationship between the organisms, this population would be promising for use against the three most important Meloidogyne spp. (M. javanica, M. incognita, M. arenaria) in Florida (R. A. Dunn, personal communication). Moreover, since this population of B. penetrans attached in greater numbers to M. javanica than to M. incognita, it may be more effective against M. javanica than M. incognita.

In the laboratory, a dosage-response experiment showed an increase in infection with increasing inoculum until a maximum in infection was reached. Then infection decreased as the amount of inoculum increased. If a similar function describes this relationship in field soil, a maximum concentration of B. penetrans may not have been reached in the present study because the infection of juveniles in soil increased during the tobacco and soybean seasons. Even though the full potential of B. penetrans in preventing the parasitism of M. incognita may not have been reached, the consistent benefit of B. penetrans in this and the previous year (CHAPTER 1) indicates that B. penetrans may be a good component of control programs where integrated techniques and a combination of pathogens are used to reduce M. incognita damage to crops.

For example, experiments using a combination of antagonists such as B. penetrans, Dactylella oviparasitica Stirling and Mankau, Paecilomyces lilacinus (Thom) Samson and Nematophthora gynophila Kerry and Crump against

Meloidogyne spp. might be practical. Dactylella oviparasitica is a parasite of M. incognita eggs (Stirling and Mankau 1978). Paecilomyces lilacinus is a parasite of M. incognita eggs and females (Jatala et al. 1980). Nematophthora gynophila is a parasite of Heterodera spp. females (Kerry and Crump 1977) and has been found parasitizing M. acronea Coetzee (Kerry and Mullen 1981). These organisms should be tested against the economically important Meloidogyne spp. If B. penetrans and N. gynophila could be produced on a large scale, extensive field testing could be initiated. Perhaps organisms already described should be evaluated alone and in combination with other organisms, and the search for other pathogens should be continued.

Summary

The pathogenicity of Bacillus penetrans to Meloidogyne incognita was tested under field conditions. Bacillus penetrans was added to concrete-bordered plots infested with M. incognita and B. penetrans into which tobacco and soybeans were planted in sequence. Both crops showed trends of increased yields with decreasing pathogenicity of M. incognita. Yields from plots infested with M. incognita and treated with B. penetrans were 24% greater for tobacco and 35% greater for soybeans than those from plots infested with M. incognita alone. Soil samples taken at the harvest of soybeans revealed fewer second-stage-juvenile nematodes in plots infested with B. penetrans than the nematode-infested plots without B. penetrans. Spores from this population of B. penetrans attached to M. javanica, M. incognita and M. arenaria.

CHAPTER 3
EFFECT OF Bacillus penetrans ON MOVEMENT OF AND
ROOT PENETRATION BY Meloidogyne incognita

Introduction

Possible modes of action (impaired motility, reduced root penetration and reduced natality) of Bacillus penetrans to Meloidogyne spp. were suggested in previous studies (Prasad 1971, CHAPTERS 1, 2). The purpose of the studies reported in this chapter was to determine the effects of B. penetrans on root penetration by and movement of M. incognita.

Materials and Methods

Root Penetration by Meloidogyne incognita
Infected with Bacillus penetrans

The effect of B. penetrans on the penetration of M. incognita into tomato roots (Lycopersicon esculentum Miller 'Rutgers') was investigated using a modified penetration inhibition test (Bunt 1975). Ten cubic centimeters of quartz sand (99.3% sand, 0.3% silt and 0.4% clay with a sand particle size distribution of 22% 1-0.5 mm, 41.4% 0.5-0.25 mm, 35.4% 0.25-0.1 mm and 0.5% 0.1-0.05 mm) and 4 ml of water were added to 25-ml glass vials into which 50 juveniles of M. incognita in water were injected. There were two treatments: juveniles infected with B. penetrans and juveniles not infected (control). Each treatment was replicated 25 times.

The infected juveniles were obtained by adding healthy juveniles to 100 cm³ of B. penetrans-infested potting soil (90.6% sand, 3.9% silt and

5.5% clay) that had been moistened and incubated for 3 days at 28 C prior to the addition of nematodes. Then the juveniles were extracted from the soil by modifications (CHAPTER 1) of the sieving and centrifugation method of Jenkins (1964). The juveniles used for the controls were treated identically except that they were added to soil not infested with B. penetrans. A random sample of 30 juveniles from each treatment was examined (at 200X) to determine the number of attached spores before adding juveniles to the vials. Twenty-five juveniles from the infested soil had more than 20 spores attached to their cuticles. Of the remaining five juveniles, one had 13 spores, one had 10 spores and three had one spore each. None of the 30 juveniles from noninfested soil had spores on their cuticles.

Twenty-four hours after infesting the vials with M. incognita, tomato seedlings were planted in each vial. The vials were placed randomly in an incubator at 28 C and a cycle of 13 hr light (260 Lux) and 11 hr dark. After 7 days the galls on the roots were counted, and the effect on the formation of galls by nematodes parasitized by B. penetrans was tested with the Wilcoxon rank sum test (Hollander and Wolfe 1973).

Immediately after counting the galls the seedlings were transplanted into 10-cm-diam clay pots containing potting soil and allowed to develop further in a greenhouse. After 14 days in the greenhouse, the seedlings were removed from the pots, and the root systems were stained with acid fuchsin in lactophenol to aid in counting the nematodes (Southey 1970). This procedure was undertaken to corroborate that the galls were formed by nematodes in established feeding sites.

The effect of B. penetrans on root penetration by M. incognita was investigated further under greenhouse conditions. Tomato plants were

inoculated with healthy or infected juveniles, which were obtained as described previously. In three experiments, which were carried out for either 14 or 30 days, either 35 or 325 juveniles were added to the six pots of each treatment (Table 3.1).

At the end of the experiments, the plants were removed from the pots and when 325 juveniles were used a 4-g random sample of roots was stained with acid fuchsin in lactophenol. When 35 juveniles were used, the entire root system was stained. The numbers of nematodes within the roots in the two treatments were compared with the Wilcoxon rank sum test.

Movement of *Meloidogyne incognita* Infected with *Bacillus penetrans*

The effect of *B. penetrans* on the movement of *M. incognita* was investigated in polyvinyl chloride (PVC) tubes filled with 50 g of quartz sand or potting soil (Table 3.2). One-half inch PVC tubes (18-mm diam) were cut and glued into 0.5-in PVC 90° elbows. In half of the tubes, polyester screen with 10- μ m openings was glued onto the ends of the tubes before they were glued into the elbows. The tubes then were cut to fit flush into a 0.5-in PVC coupling. The resulting tube had an internal diameter of 18 mm and a length of 105 mm from center to center of the elbow openings.

A tomato seedling was planted into the soil at the end of the tube nearest the polyester screen. The screen restricted passage of roots and nematode juveniles. The tubes were placed horizontally for 2, 3 or 6 days in an incubator as described previously. Infected or noninfected juveniles then were injected into the end of the tube farthest from the screen and the tubes were incubated for 7 days. Each treatment was replicated four times.

Table 3.1 - The effect of number of juveniles and number of juveniles with spores of Bacillus penetrans on root penetration by Meloidogyne incognita.

<u>Expt.</u>	<u>Duration (days)</u>	<u>Number of juveniles/pot</u>	<u>Number of spores/juvenile*</u>	<u>Number of juveniles</u>
1	30	325	0	10
			1	8
			2	3
			5	1
			10	1
			12	1
			15	1
			16	1
	>20	4		

2	14	325	0	20
			1	4
			2	2
			3	1
			4	1
			5	1
			3	1

3	14	35	0	12
			1	10
			2	1
			3	1
			4	1
			9	1
			12	1
			16	1
			>20	2

* No spores were observed on juveniles from the control.

Table 3.2 - The effect of number of juveniles and number of juveniles with spores of Bacillus penetrans on the movement of Meloidogyne incognita.

Expt.	Soil type	Days seedlings present before juveniles	Number of juveniles/tube	Number of spores/juvenile*	Number of juveniles
1	sand	2	1500	3	1
				5	1
				7	1
				11	1
				>20	26

2	sand	2	600	0	11
				1	4
				2	3
				3	1
				6	1
				9	3
				10	2
				15	2
>20	3				

3	sand	3	1500	0	14
				1	5
				2	5
				3	3
				5	1
				6	1
>20	1				

4	potting	6	3000		

* No spores were observed on juveniles from the control.

The infected juveniles were obtained as described previously except for the last experiment in which potting soil infested and not infested with B. penetrans was used. In this experiment, noninfected-second-stage juveniles not older than 2 days were added to the tubes to determine whether the previous extraction process or quartz sand were inhibiting the movement of juveniles.

After 7 days the juveniles in each half of the tube were extracted from the soil, and the proportions of juveniles from each treatment moving to the half of the tube with the tomato seedling were compared by normal approximation. When sand was used in the tubes, the nematodes were extracted by mixing the sand with water and decanting into a Millipore Swinnex-25 with a polyester screen of 10- μ m openings. When 1500 juveniles per tube were used, the screen was washed in 10 ml of water. When 600 juveniles per tube were used, the screen was washed in 5 ml of water. In each case the nematodes in a 1-ml sample were counted in a Peters counting slide. When potting soil was used in the tubes, the nematodes were extracted by sieving and centrifugation.

Results

In the penetration experiment in the laboratory, the Wilcoxon rank sum test indicated fewer galls on the roots of tomato plants inoculated with second-stage juveniles of M. incognita infected with B. penetrans (Fig. 3.1) than on tomato plants inoculated with noninfected juveniles (Table 3.3). The attained significance level of the test was $P < .001$ (the attained significance level of a test is the probability of observing a value of the statistic at least as extreme as the value observed). The

Figure 3.1 - Spores of Bacillus penetrans attached to the cuticle of a second-stage juvenile of Meloidogyne incognita.

Table 3.3 - Number of galls on tomato roots 7 days after inoculation with 50 juveniles of Meloidogyne incognita noninfected (control) and infected with Bacillus penetrans.

Control		Infected	
<u>Number of galls/plant</u>	<u>Frequency</u>	<u>Number of galls/plant</u>	<u>Frequency</u>
0	6	0	17
1	4	1	7
2	4	3	1
3	5		
4	1		
5	2		
6	1		
7	2		

total number of galls formed on the roots after 7 days was 10 for the infected nematodes and 61 for the control nematodes; after 14 days, the number of infected nematodes in the roots was nine compared with 101 noninfected nematodes.

In two of three penetration experiments in the greenhouse, fewer infected than noninfected nematodes developed in tomato roots (Experiment 1, $P = .066$; Experiment 3, $P = .004$) (Table 3.4). No difference could be claimed between the number of infected and noninfected nematodes in tomato roots in Experiment 2 ($P = .197$).

In the movement experiments, the proportion of infected juveniles moving to the half of the tube with the tomato seedling was less than that of the noninfected juveniles in Experiment 1 ($P < .001$) (Table 3.5). In the remaining experiments there was insufficient evidence to enable one to declare a smaller proportion of infected juveniles than noninfected juveniles moving to the half of the tube containing the tomato seedling (Experiment 2, $P = .92$; Experiment 3, $P = .90$; Experiment 4, $P = .40$).

Of the samples of juveniles from the infected populations that were recovered from the half of the tube containing a plant, two of 20 were infected with B. penetrans (one with 15 spores and the other with five spores).

Discussion

The decrease in root penetration of juveniles of M. incognita when infected with B. penetrans establishes one effect of B. penetrans on nematodes (Tables 3.3, 3.4). Prasad (1971) reported that greenhouse tomatoes inoculated with M. incognita had fewer galls on roots when grown in soil containing B. penetrans; juveniles of M. javanica that were heavily

Table 3.4 - Numbers of Meloidogyne incoognita in greenhouse tomato roots 14 or 30 days after inoculation with juveniles not infected (control) and infected with Bacillus penetrans.

<u>Expt.</u>	<u>Control</u>	<u>Infected</u>
1	28*	8
	38	24
	38	32
	41	32
	61	38
	62	53

2	30*	1
	34	25
	35	36
	59	41
	63	45
	67	58

3	3**	0
	6	0
	9	1
	9	1
	13	2
	18	7

* Nematodes/4 g of roots.

** Nematodes/root system.

Table 3.5 - Proportions of juveniles of Meloidogyne incoqnita not infected (control) and infected with Bacillus penetrans that moved more than 5.25 cm toward a tomato plant.

<u>Expt.</u>	<u>Control</u>	<u>Infected*</u>
1	12/164	1/164
2	7/183	14/201
3	1/259	3/173
4	4/202	2/120

* Only two of the 20 juveniles that moved more than 5.25 cm toward a tomato plant had spores attached to the cuticle; one had 15 spores and the other had five spores. Both juveniles were observed in Experiment 2.

infected with B. penetrans formed four to six galls on tomato roots after 60 days in a greenhouse, while noninfected juveniles produced numerous galls. In other treatments of Prasad's study, juveniles not heavily infected (not quantified) produced a moderate number of galls on tomato roots. This experiment suggests that the inhibition of root penetration is related to the number of spores attached to a nematode.

The relationship of the number and degree of infected juveniles in a population and the inhibition of root penetration needs further investigation. Also, when infected juveniles establish feeding sites in root tissue, the effects of B. penetrans on developing nematodes (such as uptake of nutrients, natality and longevity) need to be quantified.

Reduced movement toward plant roots by juveniles of M. incognita when infected with B. penetrans (Table 3.5) may explain in part the inhibition of root penetration by M. incognita (Tables 3.3, 3.4). Prasad (1971) demonstrated that the number of juveniles of M. javanica extracted from soil containing B. penetrans was less than that from soil without the bacterium. He collected 279 of 2000 M. javanica from Baermann funnels containing soil infested with B. penetrans compared with 1719 of 2000 from soil without the bacterium. One possible explanation of these results is impaired motility of the juveniles after infection with B. penetrans. Additional experiments should be carried out to determine the causes of the reduced directed movement of nematodes infected with B. penetrans. Two possible effects of B. penetrans on directed movement of M. incognita might be impaired motility and disorientation.

The proportions of noninfected juveniles of M. incognita that moved 5 to 10 cm toward tomato plants (Table 3.5) were smaller than those reported in a previous study of horizontal movement of M. javanica.

Prot (1976) demonstrated that approximately 40% of juveniles of M. javanica penetrated tomato roots in 5 days when placed 10 cm horizontally from them. The author was unable to determine the reasons for the poor movement of M. incognita in the present studies. Although movement was poor in these experiments, most of the nematodes in both treatments that moved toward the tomato plants were not infected. This observation suggests that movement of infected juveniles is impaired.

Summary

Laboratory and greenhouse experiments were conducted to determine the effect of B. penetrans on root penetration and movement of M. incognita. In a laboratory experiment, juveniles of M. incognita infected with B. penetrans caused fewer galls (a total of 10) to form on the roots of tomato plants than did noninfected juveniles (a total of 61). In two greenhouse experiments B. penetrans reduced the number of nematodes in tomato roots. In a third experiment there was insufficient evidence to declare a lower number of nematodes in tomato roots inoculated with juveniles infected with B. penetrans than in roots inoculated with noninfected juveniles. In one of four laboratory experiments B. penetrans reduced the percentage of juveniles moving toward tomato plants from seven to one. In the remaining three experiments there was insufficient evidence to claim that B. penetrans reduced the directed movement of M. incognita.

DISCUSSION AND CONCLUSIONS

The increased crop yields in four of five experiments resulting from adding B. penetrans to plots containing an established population of M. incognita, demonstrated that B. penetrans reduced the pathogenicity of the population of M. incognita. The effect of B. penetrans on M. incognita now can be extended from greenhouse conditions (Prasad 1971) to field conditions.

The proportion of M. incognita juveniles with spores on the cuticle increased through the field experiments. It is conjectured that this increase in incidence of infection indicates that the number of B. penetrans in the soil increased. The addition of B. penetrans at the beginning of the second year of experiments (CHAPTER 2) may have contributed to the increased incidence of infection of juveniles in soil. Alexander (1981) reviewed studies of the addition of bacteria to natural ecosystems that suggested that for bacteria that persist in soil, there is a characteristic number that is maintained. Determining this characteristic number for B. penetrans in agricultural soil and, in turn, the optimum effect of that concentration of B. penetrans on a population of plant-parasitic nematodes would be an interesting continuation of the present study.

A dosage-response experiment in the laboratory showed an increase in infection of juveniles with increasing inoculum until a maximum in infection was reached. Then infection decreased as the amount of inoculum increased. If a similar function describes this relationship in field soil, a maximum concentration of B. penetrans may not have been reached

in the present study because of the continued increase in the incidence of infection of juveniles during the experiments. Even though the full potential of B. penetrans in preventing the parasitism of M. incognita may not have been reached, the consistent benefit of B. penetrans during the two growing seasons indicates that B. penetrans may be a good component in a control program where pathogens, resistant varieties, crop rotations and other techniques are used to reduce M. incognita damage to crops.

Experiments using combinations of antagonists such as B. penetrans, Dactylella oviparasitica Stirling and Mankau, Paecilomyces lilacinus (Thom) Samson and Nematophthora gynophila Kerry and Crump against Meloidogyne spp. should be undertaken. Dactylella oviparasitica is a parasite of M. incognita eggs (Stirling and Mankau 1978). Paecilomyces lilacinus is a parasite of M. incognita eggs and females (Jatala et al. 1980). Nematophthora gynophila is a parasite of Heterodera spp. females (Kerry and Mullen 1981). These organisms need to be tested against the economically important Meloidogyne spp., and efforts to culture B. penetrans and N. gynophila should be initiated. Perhaps organisms already described should be reevaluated as well as continuing the search for biological control organisms.

A tentative conclusion that B. penetrans reduces the population size of M. incognita could be drawn from the samples of juveniles in soil at the midseason of tobacco (1981) and at the end of the soybean season (1982). Two of three greenhouse experiments also supported this conclusion. However, the size of the population at a given time is dependent on so many factors that a generalization is difficult to make. For example, a high initial infestation of juveniles may damage a host to a degree that

subsequent lack of food limits the increase in nematode numbers. On the other hand, if juveniles are in some way inhibited from penetrating roots or developing once within the roots, damage to the host may be less and juveniles that do successfully complete their life cycle may produce more offspring due to less competition. As a result of this early protection of the host, the size of the population may increase later in the season.

The decrease in root penetration by juveniles of M. incognita when infected with B. penetrans establishes one effect of B. penetrans on nematodes (CHAPTER 3). These results agree with Prasad (1971) who reported that greenhouse tomatoes inoculated with M. incognita had fewer galls on roots when grown in soil containing B. penetrans than when grown in soil without B. penetrans and that juveniles of M. javanica that were heavily infected with B. penetrans formed less than six galls on tomato roots after 60 days in a greenhouse, while noninfected juveniles produced numerous galls. The relationship of the number and degree of infected juveniles in a population and the inhibition of root penetration needs to be investigated. Also when infected juveniles establish feeding sites in root tissue, the effects of B. penetrans on developing nematodes (such as uptake of nutrients, natality and longevity) need to be quantified.

The reduced movement toward plant roots of juveniles of M. incognita when infected with B. penetrans (CHAPTER 3) may explain in part the inhibition of root penetration of M. incognita. The proportions of noninfected juveniles of M. incognita that moved 5 to 10 cm toward tomato plants were smaller than those reported in a previous study of horizontal movement of M. javanica. Prot (1976) demonstrated that approximately 40% of the juveniles of M. javanica penetrated tomato roots in 5 days when placed 10 cm horizontally from the tomato plants. I was unable to determine

the reasons for the poor movement of M. incognita in the present studies. Prasad (1971) demonstrated that the number of M. javanica extracted from soil containing B. penetrans was substantially less than that from soil without B. penetrans. One possible explanation of these results is impaired motility of the juveniles after infection with B. penetrans. Additional experiments should be carried out to determine the importance of reduced directed movement of nematodes infected with B. penetrans.

The population of B. penetrans used in this study also attaches to M. javanica and M. arenaria. Assuming that attachment of spores indicates a host-pathogen relationship between the organisms, this population would be promising for use against the three most important Meloidogyne spp. (M. javanica, M. incognita, M. arenaria) in Florida (R. A. Dunn, personal communication). Moreover, since this population of B. penetrans attached more readily to M. javanica than to M. incognita, it might be possible to use it more effectively against M. javanica than M. incognita.

LITERATURE CITED

- Alexander, M. 1977. Soil microbiology. 2nd ed. John Wiley & Sons, New York. 467 p.
- Alexander, M. 1981. Why microbial predators and parasites do not eliminate their prey and hosts. *Ann. Rev. Microbiol.* 35:113-133.
- Allen, M. W. 1957. A new species of the genus Dolichodorus from California (Nematoda: Tylenchida). *Proc. Helminthol. Soc. Wash.* 24: 95-98.
- Bergey's Manual of Determinative Bacteriology. 1974. 8th ed. R. E. Buchanan and N. E. Gibbons, eds. Williams & Wilkins Co., Baltimore. 1268 p.
- Bunt, J. A. 1975. Effect and mode of action of some systemic nematocides. *Meded. Landbouwhogeschool, Wageningen.* 75-10:1-127.
- Cobb, N. A. 1906. Fungus maladies of the sugarcane, with notes on associated insects and nematodes. Hawaiian Sugar Planters Assoc. Expt. Sta. Div. Path. Physiol. Bull. 5:153-195.
- Cross, T. 1970. The diversity of bacterial spores. *J. Appl. Bacteriol.* 33:95-102.
- Cross, T., P. D. Walker, and G. W. Gould. 1968. Thermophilic Actinomycetes producing resistant endospores. *Nature* 220:352-354.
- Davidson, E. W. 1973. Ultrastructure of American foulbrood disease pathogenesis in larvae of the worker honey bee, Apis mellifera. *J. Invert. Pathol.* 21:53-61.
- Dorokhova, L. A., N. S. Agre, L. V. Kalakoutskii, and N. A. Krassilnikov. 1968. Fine structure of spores in a thermophilic actinomycete Micromonospora vulgaris. *J. Gen. Appl. Microbiol.* 14:295.
- Dutky, E. M. 1978. Some factors affecting infection of plant parasitic nematodes by a bacterial spore parasite. M.S. Thesis. Univ. of Maryland, College Park. 44 p.
- Franklin, M. T. 1979. Economic importance of Meloidogyne in temperate climates. Pages 331-339 in F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (Meloidogyne species) systematics, biology and control. Academic Press, New York. 477 p.

- Hollander, M., and D. A. Wolfe. 1973. Nonparametric statistical methods. John Wiley & Sons, New York. 503 p.
- Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of Meloidogyne spp., including a new technique. Plant Dis. Reprtr. 57:1025-1028.
- Jatala, P., R. Kaltenbach, M. Bocangel, A. J. Devaux, and R. Campos. 1980. Field application of Paecilomyces lilacinus for controlling Meloidogyne incognita on potatoes. J. Nematol. 12:226-227. (Abstr.)
- Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Reprtr. 48:692.
- Kawanishi, C. Y., C. M. Splittstoesser, and H. Tashiro. 1978. Infection of the European chafer, Amphimallon majalis, by Bacillus popilliae: Ultrastructure. J. Invert. Pathol. 31:91-102.
- Kepner, J. L., and D. H. Robinson. 1982. A distribution-free rank test for ordered alternatives in randomized complete block designs. Tech. Rep. No. 176, Department of Statistics, Univ. of Florida.
- Kerry, B. R., and D. H. Crump. 1977. Observations on fungal parasites of females and eggs of the cereal cyst-nematode, Heterodera avenae, and other cyst nematodes. Nematologica 23:193-201.
- Kerry, B. R., and L. A. Mullen. 1981. Fungal parasites of some plant parasitic nematodes. Nematropica 11:187-189.
- Lamberti, F. 1979. Economic importance of Meloidogyne spp. in subtropical and Mediterranean climates. Pages 341-357 in F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (Meloidogyne species) systematics, biology and control. Academic Press, New York. 477 p.
- Mankau, R. 1975. Bacillus penetrans n. comb. causing a virulent disease of plant-parasitic nematodes. J. Invert. Pathol. 71:333-339.
- Mankau, R., J. L. Imbriani, and A. H. Bell. 1976. SEM observations on nematode cuticle penetration by Bacillus penetrans. J. Nematol. 8:179-181.
- Neter, J., and W. Wasserman. 1974. Applied linear statistical models. Richard D. Irwin, Inc., Homewood, IL. 842 p.
- Prasad, N. 1971. Studies on the biology, ultrastructure and effectiveness of a sporozoan endoparasite of nematodes. Ph.D. Dissertation. Univ. of California, Riverside. Available from: University Microfilms, Ann Arbor, MI; Publication No. 72-10,388. 108 p.
- Prot, J. C. 1976. Amplitude et cinétique des migrations du nématode Meloidogyne javanica sous l'influence d'un plant de tomate. Cah. ORSTOM, Ser. Biol. 11:157-166.

- Sasser, J. N. 1979. Economic importance of Meloidogyne in tropical countries. Pages 359-374 in F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (Meloidogyne species) systematics, biology and control. Academic Press, New York. 477 p.
- Sasser, J. N. 1980. Root-knot nematodes: A global menace to crop production. Plant Dis. 64:36-41.
- Sayre, R. M., and W. P. Wergin. 1977. Bacterial parasite of a plant nematode: Morphology and ultrastructure. J. Bacteriol. 129:1091-1101.
- Slack, J. M., and M. A. Gerencser. 1975. Actinomyces, filamentous bacteria: Biology and pathogenicity. Burgess Publishing Co., Minneapolis. 158 p.
- Sokal, R. R., and F. J. Rohlf. 1981. Biometry. 2nd ed. W. H. Freeman & Co., San Francisco. 776 p.
- Southey, J. F., ed. 1970. Laboratory methods for work with plant and soil nematodes. Tech. Bull. 2. Ministry of Agriculture, Fisheries and Food, London. 148 p.
- Splittstoesser, C. M., C. Y. Kawanishi, and H. Tashiro. 1975. Germination and outgrowth of Bacillus popilliae in hemolymph slide mounts. J. Invert. Pathol. 25:371-374.
- Splittstoesser, C. M., C. Y. Kawanishi, and H. Tashiro. 1978. Infection of the European chafer, Amphimallon majalis, by Bacillus popilliae: Light and electron observations. J. Invert. Pathol. 31:84-90.
- Stirling, G. R., and R. Mankau. 1978. Parasitism of Meloidogyne eggs by a new fungal parasite. J. Nematol. 10:236-240.
- Stirling, G. R., and M. F. Wachtel. 1980. Mass production of Bacillus penetrans for the biological control of root-knot nematodes. Nematologica 26:308-312.
- Thorne, G. 1940. Duboscqia penetrans, n. sp. (Sporozoa, Microsporidia, Nosematidae), a parasite of the nematode Pratylenchus pratensis (de Man) Filipjev. Proc. Helminthol. Soc. Wash. 7:51-53.

BIOGRAPHICAL SKETCH

Stephen Michael Brown was born in Glendale, California, in 1946. He received a Bachelor of Science degree with distinction in zoology and a Master of Arts degree in biology from San Diego State University with the first year of his graduate studies spent at the Free University of Berlin, West Germany.

After teaching for several years, he was hired by the United Fruit Company to study the plant-parasitic nematodes of banana in Central America. This position brought about his matriculation at the University of Florida to pursue a Doctor of Philosophy degree in nematology.

Stephen is a member of the American Phytopathological Society, the American Statistical Association, the National Audubon Society, the Organization of Tropical American Nematologists, the Sierra Club, Sigma Xi, the Society of Nematologists and the Southeastern Biological Control Working Group.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Grover C. Smart, Jr., Chairman
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Don W. Dickson
Don W. Dickson
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert P. Esser
Robert P. Esser
Assistant Professor of Entomology
and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David J. Mitchell
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edward P. Previc
Associate Professor of Microbiology
and Cell Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 1983

Dean, College of Agriculture

Dean for Graduate Studies and Research

UNIVERSITY OF FLORIDA

3 1262 08553 4393