MANAGEMENT OF SOIL SUPPRESSIVENESS TO PEANUT ROOT-KNOT NEMATODE USING *Pasteuria penetrans*

By

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

UNIVERSITY OF FLORIDA

2006
To My Mom and Dad
ACKNOWLEDGEMENTS

I am indebted for the assistance that I received from many people during the execution of this study. First and foremost I wish to express my deepest appreciation to Dr. Don Dickson, chairman of my committee, for awarding me the assistantship to undertake this study. I wish also to thank him for agreeing to be my major advisor. Without the help and the support I received from him, this study would have been very difficult to achieve. I also express my gratitude to the other members of my committee, Drs. Leon Allen, Jr., James F. Peston, Janete Brito, and Oscar Liburd, for their support, suggestions, encouragements, and friendship.

I would like to thank the Kenya government through the Director, Kenya Agricultural Research Institute (KARI), for giving me the opportunity to work for them and providing me with a study leave that enabled me to pursue this study.

My sincere thanks go to Dr. Dickson’s staff and members of his laboratory, Dr. Maria Mendes, Luis Corlozo, and Timothy Sheffield, for helping me with research at Citra, in the laboratory and greenhouse. I also thank my fellow colleagues Marisol Davila, Jon Hamill, Ashley Johnson, Heather Smith, Joey Orajey and Marco Cordero for their assistance and friendship during the course of this study.

Sincere thanks go to Mrs. Debbie Hall who guided me to follow the university and departmental rules throughout the program, and made sure that I met all the deadlines.
Special thanks go to my wife Lillian and my son Victor for their love, support, and encouragement during the course of the study. Lillian worked very hard when I was still in Kenya to ensure that I could come to the United States for my studies.
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Pasteuria penetrans (Thorne) Sayre & Starr is known to contribute to soil suppressiveness against root-knot nematodes in many microplot and field studies. To understand the nature of the suppressiveness the effect of different \( P. \) penetrans concentrations on attachment, penetration and fecundity were evaluated in the laboratory. Different attachment levels were achieved using different ratios of second-stage juveniles (J2) to endospores. As the mean attachment level per J2 increased, the percentage of J2 penetrating host plant roots decreased. As few as 3.5 endospore/J2 reduced the ability of Meloidogyne arenaria to enter host roots. Egg mass numbers were less when the endospore attachment was high but galling indices were not affected by endospore attachment. Thus, \( P. \) penetrans-induced suppressiveness is manifested in the form of limited penetration and egg mass production. A field study was conducted to determine whether \( P. \) penetrans could be transferred to a new site and become established to levels that would be suppressiveness to \( M. \) arenaria. 1,3-D and chloropicrin were applied each
year before planting peanut. 1,3-D effectively reduced root-knot nematodes thereby decreasing the build-up of *P. penetrans*, whereas chloropicrin prevented endospore development within root-knot nematode females also decreasing the build-up of *P. penetrans*. After two peanut crop seasons the greatest increase of *P. penetrans* was recorded in nontreated plots (*Pasteuria* treated only). Microplots were used to determine the effect of summer host crops (long-season peanut crop vs. short-season squash crop) and addition of different levels of *M. arenaria* J2 on development of *P. penetrans*. The most effective tactic for amplifying *P. penetrans* was to add 40 J2 per 100 cm$^3$ of soil and to plant a long-season crop such as peanut.
CHAPTER 1
INTRODUCTION

Peanut Production

The cultivated peanut (Arachis hypogaea L.), also called goober, pindar, groundnut or earthnut, is an annual, self-pollinating, herbaceous legume native to South America. It is a member of the pea family (Aerts and Nesheim, 2001; Hammons, 1982), and is characterized as a geotropic plant that produces pods underground. Flowering begins 4 to 6 weeks after planting and extends over a period of several weeks. One week after the flowers are fertilized, pegs develop and grow into the soil 2 to 7 cm deep (Dickson and De Waele, 2005; Whitty, 2002). Once in the soil, the fertilized ovaries, located behind the tip of the peg, enlarge rapidly and pod growth begins. Two to four seeds are formed within a pod. The number of seeds formed per pod depends on the peanut variety. The length of time necessary for pod development to maturity varies with variety and environmental conditions (Dickson and De Waele, 2005).

Peanut is one of the world’s major oil-seed crops and is listed as one of the 20 crop plants that stand between man and starvation (Wittwer, 1981). Seeds from peanut are rich in calories and contain 25% protein. Most of the peanuts in the United States are grown for peanut butter, peanut candy, roasted peanuts, peanut oil and other peanut products (Whitty, 2002). In home gardens, peanut may be grown for boiling, roasting, eating raw or other uses. Sometimes peanut pods are rooted from the soil by hogs in a process commonly called “hogging off.” The vines of peanut can also be harvested for hay (Whitty, 2002).
Currently, peanut is cultivated on all six continents, with major production in over 32 countries (Dickson and De Waele, 2005). Eight countries, the People’s Republic of China, India, the USA, Indonesia, Argentina, Senegal, Zaire and Myanmar produce 72% of the world supply. In 2003, approximately 31.6 metric tonnes were produced on 22.4 million hectares. The highest average yield per hectare are produced in the United States (2.81 t/ha), followed by the People’s Republic of China (2.59 t/ha), whereas other countries have much lower yields (Dickson and De Waele, 2005).

Peanut production is concentrated in three major geographic regions of the United States: the southeast, Virginia and the Carolinas, and the southwest (Anonymous, 2005). The southern United States accounts for 64% of the total United States peanut production. Peanut is considered a major crop in this region (Minton and Baujard, 1990). The $4 billion United States peanut industry employs an estimated 150,000 people on-farm, shelling, and in manufacturing plants. The majority of the employment occurs in rural areas. Because of the concentration of the peanut industry and jobs in the rural communities of producing states, the economic impact of peanut is magnified in these regions (Bridges et al., 1994).

There are four major market types of peanut: Virginia, Runner, Spanish, and Valencia. In the southeastern United States, Florunner, a runner type peanut, was commonly planted beginning in the 1960s and continued through the early 1990s. The planting of this cultivar, which is highly susceptible to tomato spotted wilt virus, was curtailed when the disease became a major problem for peanut producers (Whitty, 2002). The cultivar is being replaced by several newer cultivars that are resistant to the disease. Almost all peanut planted in Virginia and the Carolinas are the Virginia type, whereas in
the southwest, Spanish (55%), Runner (42%), and Valencia (3%) types are grown. New Mexico produces mostly Valencia type (Anonymous, 2005; Whitty, 2002).

Florida ranks 6th nationally in peanut production, producing approximately 6% of the total crop produced in the United States. Average state yields generally fluctuate between 2,578 to 3,363 kg/ha, with a potential harvest of up to 6,726 kg/ha on selected fields (Bridges et al., 1994). Cash receipts from Florida’s peanut crop totaled over $61 million in 1999 and ranged from $69.1 million to $52.4 million during the previous years (Aerts and Nesheim, 2001; Anonymous, 1998; 2000; Bridges et al., 1994). Expected annual returns are generally in the range of $1,977 to $2,965/ha (Jordan, 1999). Pest management accounts for a large portion of the variable cost of producing peanut. Estimated crop production costs are approximately $1,877/ha, based on a 3,363 kg/ha yield in the southeastern United States (Aerts and Nesheim, 2001). More than 36% of the total operating costs are invested in pesticides.

Plant-pathogenic nematodes, namely *Meloidogyne* spp., *Pratylenchus brachyurus*, *Belonolaimus longicaudatus*, *Criconemoides ornatus*, *Aphelenchoides arachidis*, *Aphasmatylenchus straturatus*, *Scutellonema cavenessi*, *Tylenchorhynchus brevilineatus*, and *Ditylenchus africanus* are primary disease-inducing agents of peanut; however, several of these are pathogens of localized importance whereas others are distributed worldwide (Dickson and De Waele, 2005). Based on a worldwide survey by nematologists, annual losses caused by all nematodes to peanut were estimated at 12% and monetary losses were estimated at US$1.03 billion (Sasser and Freckman, 1987).

**Root-knot Disease of Peanut**

*Meloidogyne* spp. are placed in the class Secernentea, order Tylenchida, family Heteroderidae, subfamily Meloidogyninae, genus *Meloidogyne*. The genus *Meloidogyne*
was first described in 1855 by Berkeley as the causal agent of root-knot diseases of cucumber in England (Thorne, 1961). Throughout the subsequent years, studies revealed many physiological and biological differences among field populations of these nematodes (Christie, 1946; Christie and Albin, 1944). This led B. G. Chitwood to revise the genus in 1949 based on morphological differences. He recognized five species and one variety (Netscher and Sikora, 1990): *Meloidogyne arenaria* Neal, (1889), *M. exigua* Göldi (1982), *M. hapla* Chitwood (1949), *M. incognita* Kofoid and White (1919), *M. incognita* var. acrita, and *M. javanica* Treub (1885).

Recently, the updated number of described species of *Meloidogyne* was reported as 80, 50% of which have been reported during the last 20 years (Karssen, 2002). A survey conducted by the scientists involved in the International *Meloidogyne* Project (IMP) in 75 countries reported that *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* were among the most common and economically important species of root-knot nematodes in agricultural soils (Netscher and Sikora, 1990).

All of these species except *M. incognita* are known to infect peanut and each is capable of causing severe suppression of peanut yields and fruit quality (Dickson and De Waele, 2005). Two of the species, *M. arenaria* (peanut root-knot nematode) and *M. javanica* (Javanese root-knot nematode) are highly virulent pathogens of peanut, whereas *M. hapla* (northern root-knot nematode) causes less damage but nonetheless is an important disease-inducing agent of peanut. A new *Meloidogyne* spp. causing root-knot disease on peanut in Texas, USA, was recently described as *M. haplanaria* (Eisenback et al., 2003). Currently, little is known about the distribution or virulence of this new species in peanut producing regions of the United States.
Of the three major species that occur on peanut worldwide (Sasser, 1977), *M. arenaria* and *M. javanica* are common in warm and hot regions of the world, whereas *M. hapla* occurs only in cooler regions (Dickson and De Waele, 2005). *M. javanica* was first reported parasitizing peanuts in Zimbabwe (Martin, 1958). Although *M. javanica* is highly virulent pathogen, it is less frequently encountered on peanut in the United States than *M. arenaria*. This species is reported parasitizing peanut in Georgia (Minton et al., 1969), Texas (Tomaszewski et al., 1994), and most recently in Florida (Cetintas et al., 2003). *M. javanica* is also known to occur on peanut in Egypt (Ibrahim and El-Saedy, 1976b), Brazil (Carneiro et al., 2003; Lordello and Gerin, 1981), and India (Sakhuja and Sethi, 1985a). *M. hapla* has been reported infecting peanut in all peanut-producing states in the United States with the exception of Florida (Dickson, 1998), but this species is encountered more frequently in the northern latitudes (Dickson and De Waele, 2005). The nematode may be encountered at higher elevations in tropical regions (Eisenback and Triantaphyllou, 1991).

**Distribution and Importance of Peanut Root-knot Nematode**

The peanut root-knot nematode, *Meloidogyne arenaria*, is the most widespread and destructive nematode pathogen of peanut (Aerts and Nesheim, 2001; Dickson, 1985; Minton and Baujard, 1990; Porter et al., 1984). In the United States, this nematode is the dominant aggressive species parasitizing peanut in Alabama, Florida, Georgia, and Texas, whereas this species occurs less frequently in North Carolina, South Carolina and Virginia. In other regions of the world, *M. arenaria* is reported damaging peanut in Zimbabwe (Martin, 1958), Israel (Orion and Cohn, 1975), Egypt (Ibrahim and El-Saedy, 1976a), India (Dhurj and Vaishnav, 1981; Sakhuja and Sethi, 1985b; Sharma et al., 1978), Taiwan (Cheng and Tu, 1980; Cheng et al., 1981), and China (Zhang, 1985). This
species exists as two host races distinguished only on the basis of their reaction on peanut (Taylor and Sasser, 1978). Race 1 infects and reproduces on peanut, whereas race 2 does not. Both races are widespread in many regions where peanut is produced and they both cause disease on numerous agronomic and horticultural crops. The presence of the two host races makes the use of routine nematode advisory services difficult because of the time required to determine their identity (Dickson and De Waele, 2005).

Numerous J2, the infective stage, are generally distributed throughout the soil profile of infested fields. In deep sandy soils, the largest numbers of infective juveniles are generally found 30 to 123 cm deep at planting (Dickson and Hewlett, 1988). The J2 are mobile and can move up from the deeper soil depths to infect plant roots, but hard pans of soil with underlying clay restrict the downward movement of the nematode (Dickson and Hewlett, 1988). *M. arenaria* causes substantial yield losses at relatively low population densities. In Texas, initial population densities of 9 to 19 J2/100 cm³ of soil caused 10% suppression of yield (Wheeler and Starr, 1987), whereas the damage threshold in Florida was determined to be as low as 1 J2/100 cm³ of soil (McSorley et al., 1992).

**Life Cycle**

The life cycle of *M. arenaria* follows that of a typical root-knot nematode. The developmental life stages include an egg stage, four juvenile stages, and males and females. The first-stage juvenile develops following embryogenesis and molts within the egg shell to form the J2. The J2, which is eel-worm shaped, hatches and becomes the mobile infective stage. The J2 moves through the soil pore spaces in a film of water surrounding soil particles. They penetrate host roots, generally well behind the zone of differentiation, by repeatedly thrusting their stylet into the root surface cells.
After penetration, the nematode establishes a feeding site, commonly referred to as giant-cells, in the vascular tissue. These giant cells are specialized feeding cells that are a result of the infection process. These cells increase in size (hypertrophy) and there is a corresponding increase in number of cells surrounding the giant cells (hyperplasia). As a consequence of the hypertrophy and hyperplasia, galls or knots are formed on roots and warty-like tissue forms on pegs and pods (Dickson and De Waele, 2005). Once the J2 positions itself for continuous feeding, the nematode becomes a sedentary endoparasite and begins to enlarge in body shape. The nematode molts three more times, developing into the third and fourth-stage juveniles, and male and females. Males formed after the fourth molt become vermiform in shape, whereas the females become globose-pyriform shaped. Males are considered as free-living forms. Their numbers will increase when plants are under stress caused by drought, overcrowding, or high soil temperatures. The mode of reproduction of *M. arenaria* is primarily by pathenogenesis, however the nematode may reproduce via amphimixis. The life cycle of the nematode is completed when the female begins to lay eggs. The female deposits single-celled eggs in a gelatinous matrix at or near the root surface. Eggs are laid singly in to a gelatinous matrix that is secreted by glands located near the vulva. This results in the formation of an egg mass. The number of eggs that are laid may range from almost none under unfavorable conditions to as many as 2,000 under more favorable conditions. The position of the egg masses on the root surfaces of the galled tissue facilitates both egg hatch and secondary infection of the roots, pegs and pods by freshly-hatched juveniles. Egg masses are about 1 mm in width, and appear brownish in color when they are observed adhering to galled tissue (Dickson and De Waele, 2005). Egg masses of *M.*
arenaria may exist in large numbers on pegs, pods, and roots during the growing seasons but decrease dramatically after peanut harvest. Second-stage juveniles exist throughout the year and are the primary survival stage with the highest densities occurring after a peanut harvest (Dickson and De Waele, 2005).

Symptoms and Diagnosis

Peanut plants infected with the peanut root-knot nematode generally have noticeable above- and below-ground symptoms. Above-ground symptoms can be observed as early as 45 to 75 days after planting, but most severe symptoms are generally observed 90 to 120 days after planting (Dickson and De Waele, 2005). Above-ground symptoms of root-knot disease may be subtle or very conspicuous, especially as the crop nears maturity. The severity of symptoms depends on the growing environment as well as the population density of root-knot nematode juveniles at the time of planting. In some cases, stunting of young plants may be severe (Dickson and De Waele, 2005).

The general characteristics of diseased peanut plants are typical of other plants infected by root-knot nematodes. As the crop nears maturity, heavily infected plants may be severely stunted, showing chlorosis, incipient wilting, and nutrient deficiencies. Symptoms are distributed in patches of varying sizes. If drought occurs near the end of the peanut growing season, the severity of root-knot disease is accentuated and weakened plants die (Dickson and De Waele, 2005). Early season symptoms include stunted plants that fail to cover the soil between rows. Second-stage juveniles infect peanut plants soon after germination, but noticeable galling and egg masses are not apparent on the roots until 55 to 90 days after planting. The characteristic symptom on roots is the abnormal swellings (galls or knots), however, these are often difficult to see. But when they occur on pegs and pods they are easily discernable.
Galled tissue on roots may obtain a diameter larger than that of non-galled roots, but because of the abundance of nodules containing nitrogen-fixing bacteria (*Bradyrhizobium* sp.) the amount of galling is difficult to determine (Dickson and De Waele, 2005). Galls on peanut roots are small and generally discrete, whereas galls on other host crops such as tomato may be large and become coalesced. Nematode galls can be distinguished from nodules containing nitrogen-fixing bacteria. Nodules are distinctive, round swellings attached to the root and are easily detached, whereas nematode galls are swellings that constitute a part of the fibrous root system and cannot be removed without destroying the integrity of the root. Second-stage juveniles can infect nodules and in some instances galls may appear on the nodules (Dickson and De Waele, 2005; Minton and Baujard, 1990; Porter et al., 1984).

The nematode may also infect pegs and pods after blooming and initiation of pod set (generally 45 days after planting). Galling on pegs and pods is more distinctive than that on roots but they do not always appear even though roots are galled. Situations where galling on pegs and pods is extensive generally result in a large reduction in potential yield (Dickson and De Waele, 2005). Pegs that are heavily galled may cause the pod to abort prematurely or to drop off during harvest.

The most obvious sign of root-knot nematodes in peanut is the observation of female nematodes in galled roots, pegs and pods. Females are globose, approximately the size of a type written period on a page (800 µm length x 500 µm wide), pearly white in color, and have sharp pointed necks with the head oriented off to one side (Dickson and De Waele, 2005).
Fig. 1-1. Symptoms of peanut root-knot nematode. A) Above-ground symptoms are distributed in patches (black arrows) of varying sizes as shown in the diagram. As the crop nears maturity, heavily infected plants may be severely stunted, showing symptoms of chlorosis and incipient wilting. B) Below-ground symptoms include galled roots (red arrow) which are often difficult to see in peanut and sometimes can be confused with root nodules containing nitrogen-fixing bacteria (white arrow).
Fig. 1-2. Symptoms of peanut root-knot nematode damage on pegs and pods. The galls appear warty-like and become extensive, covering most of the pod. This is more distinctive and more easily seen than galls on the roots.
Economic Importance and Population Damage Threshold Levels

Yield suppression by plant-pathogenic nematodes is difficult to estimate because damage is seldom confined to a single nematode species (Sasser et al., 1970; 1975). Damage caused by low to moderate densities of plant-pathogenic nematodes often goes unnoticed. Where damaging levels of *M. arenaria* or *M. javanica* occur, more than 50% of the potential yield can be lost. A 100% loss has been observed in sections of severely infested fields (Dickson and De Waele, 2005). However, because of the uneven distribution of plant nematodes, losses over large fields may average less than 50%. Advisories for damaging levels of plant nematodes on peanut are usually based on numbers of juveniles in soil since most extraction procedures do not recover nematode eggs from the soil (Garcia, 1976; Rodríguez-Kábana et al., 1986). Plant nematodes may be extracted from soil collected from peanut fields. The type of nematode present and their densities determine management tactics. However, the timing when samples are collected is important in that population densities of *M. arenaria* juveniles in soil at planting time are usually relatively low or nearly undetectable but are high near or at peanut harvest. Hence, for advisory purposes it is best to determine the nematode population densities near or at peanut harvest.

Management of Peanut Root-knot Nematode

Management of the peanut root-knot nematode is required for profitable peanut production. Each peanut field should be evaluated based on the history of nematode damage before determining what management tactics to apply (Dickson and De Waele, 2005). The first line of defense should be preventing further development of the disease by reducing its spread (Dickson and De Waele, 2005). Management of root-knot disease is difficult and costly once it becomes established, both in terms of time devoted to
developing management tactics and resources that must be allocated. Tactics applied follow a similar trend of most root-knot nematode management programs. Management may involve reduction of nematode population densities or making them less infective than they would be otherwise (Johnson and Fassuliotis, 1984). The most useful methods that may be applied for peanut include chemicals and cultural practices including crop rotation. However, with the discovery and ingression of root-knot nematode resistant genes into cultivated peanut crop this may well be the most practical management tactic for the future.

**Chemical Control**

Control of root-knot nematodes through the use of nematicides is highly effective and practical, particularly on a field basis where crops of relatively high value are involved. In cases of severely infested fields, it may be the only effective choice, especially where crop rotation cannot be practiced or resistant varieties are unavailable (Dickson and De Waele, 2005; Kinloch, 2003; Rich and Kinloch, 2005).

Both fumigant and nonfumigant chemicals are available for peanut root-knot nematode control. Currently, the only fumigant nematicide remaining in the market for use on peanut is 1,3-dichloropropene (1,3-D). Fumigation with 1,3-D has been shown to be the single most effective management approach for controlling root-knot nematodes on peanut (Dickson and De Waele, 2005; Kinloch, 2003; Rich and Kinloch, 2005). The product must be applied about 7 days pre-plant to avoid possible phytotoxicity and may be applied broadcast either as a plough-down or chisel injected treatment with chisels spaced 10 to 12 inches apart. Also, an in-row treatment with a single chisel centered under the row or two chisels spaced 10 inches apart under the row provide an economical choice to broadcast application. 1,3-D is a highly volatile nematicide, thus it is important
that following application the soil be properly sealed (Dickson and De Waele, 2005; Rich and Kinloch, 2005). It is also possible to apply the product during the late autumn or early winter before seeding peanut in the following spring season (Kinloch and Dickson, 1991).

The nonfumigant nematicide, aldicarb, has been approved for several application uses for managing nematodes on peanut. In the case of fields heavily infested with root-knot nematode, a preplant broadcast or in-row application of 1,3-D may be followed by an application of aldicarb at pegging initiation (Kinloch, 2003; Rich and Kinloch, 2005). Irrespective of the mode of application growers must follow several use restrictions governing aldicarb in Florida (Rich and Kinloch, 2005).

**Crop Rotation**

Crop rotation involves the use of nonhosts or crops resistant to *M. arenaria* in rotation with peanut. Crop rotations for suppression of diseases and plant-pathogenic nematodes must include one or more years of a poor host for pathogen reproduction (Rich and Kinloch, 2005; Timper et al., 2001). When the cash value for peanut is low, this may be the only management tactic that can be used profitably (Dickson and De Waele, 2005). Plant pathogens that rely primarily on a host for proliferation will decline in numbers on a nonhost and cause less damage to the following susceptible crop (Timper et al., 2001). Rotation with resistant or nonhost crops for 2 to 3 years generally provides reasonable control of peanut root-knot nematodes. Therefore, rotation of a peanut crop with nonhosts, or less susceptible hosts to root-knot nematode is recommended for management purposes (Kinloch, 2003; Rich and Kinloch, 2005).

Grasses such as bahiagrass, bermudagrass, millet, and sorghum are among the most effective crops in reducing soil populations of peanut root-knot nematodes and should be
grown for at least 1 year before planting peanut (Dickson and De Waele, 2005; Kinloch, 2003; Starr and Morgan, 2002; Timper et al., 2001). Although some root-knot nematode reproduction is to be expected in field corn the crop may serve as an alternative to growing continuous peanut (Kinloch, 2003). Cotton is a good rotation crop with peanut in situations where a single producer grows these two crops (Dickson and De Waele, 2005). *M. arenaria, M. hapla,* and *M. javanica* do not infect cotton whereas *M. incognita* race 3 and 4 that infect cotton do not infect peanut (Rodríguez-Kábana et al., 1994; Sasser and Carter, 1982). Peanut yields were increased and densities of *M. arenaria* were reduced following 1 year of cotton (Rodríguez-Kábana et al., 1987). Generally, a 2-year monoculture of peanut and cotton is helpful in maintaining the root-knot nematode pathogens of both crops at manageable population levels (Rich and Kinloch, 2005).

Two-year rotations with cotton, bahiagrass, or velvet bean are also recommended where appropriate (Norton et al., 1977). Timper et al. (2001) reported that nematode numbers and gall indices were reduced in the peanut-cotton rotation but not in the bahiagrass-peanut rotation. A lack of nematode suppression in a bahiagrass-peanut rotation of only 2 or 3 years also was reported previously by Dickson and Hewlett (1989) and Johnson et al., (1999). A successful rotation with bahiagrass requires a longer period of greater than 3 years. Growing tropical forages, for several years has long been recognized as one of the best rotations to precede peanut (Norton et al., 1977; Rodriguez-Kabana et al., 1994). Rotations of three or more years out of peanut and other nonhosts are better than 1 or 2 year rotations (Dickson and Hewlett, 1989); however, for such a rotation to work successfully, one must manage weeds in the forage grasses. A few common weeds that occur frequently in plantings of forage grasses include hairy indigo
(Indigofera hirsute L.), alyceclover (Alysicarpus vaginalis (L) DC.), and morning-glory (Ipomoea spp.), each of which are good hosts for M. arenaria.

Crop rotation is a better tool to help keep relatively low population densities from becoming too high or for gradually reducing high population densities over several years (Dunn and Dickson, 1995). One disadvantage of crop rotation is that the suppression of nematode populations may also reduce populations of their host-specific antagonists (Timper et al., 2001). Crop rotation should not be expected to reduce a root-knot nematode population abruptly because (i) some of the nematode population will survive the winter without a host, (ii) most crop plants can support at least some nematode reproduction, and (iii) most fields have some weeds that support nematode reproduction (Dickson and De Waele, 2005).

Other Cultural Control Methods

Destruction of roots of host crops that precede peanut in a rotation interrupts nematode reproduction and will reduce the potential for damage. Ploughing soils several weeks before applying nematicides and planting peanut encourages the decay of live plant roots that protect nematodes from their enemies or from nematicides that are applied to the soil. In addition, drying soils after they have been turned will aid in the reduction of plant nematode population densities (Zhang, 1985).

Clean fallowing, though difficult to attain, is also effective at keeping peanut root-knot nematode at low densities. Peanut roots and pods should be ploughed and turned over as soon as possible after peanut harvest and air-dried for several days. This is achieved using a digger-shaker-inverter at harvest. Although some nematodes remain in the soil, this technique can work favorably with other methods like crop rotation and use
of resistant varieties because of the reduction of the initial nematode levels for the subsequent crop (Dickson, pers. comm.).

Use of Resistant Cultivars

Resistance to the peanut root-knot nematode was not available until 1999 when the cultivar COAN, with resistance to *M. arenaria* and *M. javanica*, was released by the Texas Agricultural Experimental Station (Simpson and Starr, 2001). In 2002 the cultivar NemaTAM, which has greater yield potential than COAN but with the same level of resistance to *M. arenaria* and *M. javanica* was released by the Texas Agricultural Experimental Station.

The availability of the nematode resistant cultivar gives growers an additional option for management of *M. arenaria* and *M. javanica*, and will reduce grower reliance on nematicides (Starr and Morgan, 2002). The yields of COAN and NemaTAM are greater than that of susceptible cultivars in nematode-infested soils. Both COAN and NemaTAM were developed by backcrossing nematode resistant peanut lines with the cultivar Florunner. NemaTAM was developed by completing two additional backcross generations than were used for COAN, therefore it has greater yield potential than does COAN (Church et al., 2000). Neither COAN nor NemaTAM have the yield potential of the best susceptible cultivars in the absence of root-knot nematodes. COAN was discontinued as a viable cultivar and replaced with NemaTAM.

NemaTAM is recommended only for fields known to be infested with *M. arenaria* or *M. javanica*. Since the resistance in NemaTAM is based on a single dominant gene, there is uncertainty concerning the durability of the resistance. NemaTAM is also not resistant to the tomato spotted wilt virus which causes substantial yield losses in Georgia,
Alabama and Florida (Starr and Morgan, 2002), thus the cultivar has limited utility for
the southeastern region of the United States.

**Biological Control**

Continuing environmental problems associated with the use of nematicides
(Thomason, 1987) has resulted in more scientists pursuing alternative nematode
management strategies (Kerry, 1990). Progress has been made over the past 20 years in
the identification of possible biological control organisms that offer exciting possibilities
for the future management of plant-parasitic nematodes. Biological control of nematodes
is defined as a reduction of nematodes by the action of living organisms (other than
nematode-resistant plants) that occur naturally (Stirling, 1991). Biological control offers
an alternative or supplemental management tactic to chemical or cultural control of the
peanut root-knot nematode.

**Nematode Parasite: Pasteuria penetrans**

**Historical Background**

Among the biocontrol agents studied, *Pasteuria penetrans* (Thorne) Sayre & Starr
has been recognized as having great potential for the biological control of *M. arenaria*
(Dickson et al., 1994; Minton and Sayre, 1989).

*Pasteuria penetrans* is a gram positive, endospore forming bacterium that is an
Metchnikoff (1888) was the first to designate an organism to the genus *Pasteuria*
(Metchnikoff 1888) when he discovered a parasite of water fleas, *Daphnia magna*, and
named it *Pasteuria ramosa* (Metchnikoff 1888) Sayre, 1993. The relationship of *P.
ramosa* (Metchnikoff 1888) to bacterial parasite of nematodes was not recognized until
later on. The acknowledgment of this relationship evolved from observations of soilborne organisms that were parasites of plant-parasitic nematodes.

Since this endospore forming bacteria was first discovered it has undergone numerous scientific placements and name changes. In 1940, a parasite that is now considered as Pasteuria was reported as a sporozoan parasite of Pratylenchus penetrans (Thorne, 1940). After reviewing the description, the organism described by Thorne was considered as a prokaryote (Mankau and Imbriani, 1975). Electronic microscopic examination of a similar bacterial parasite of root-knot nematodes established that structural features of the spores were similar to endospores of Bacillus spp. This led to their designation of the organism as Bacillus penetrans (Mankau, 1975b). B. penetrans differed from other members of the genus Bacillus with respect to spore shape and vegetative growth, and the ingestion of spores by nematodes was not observed (Mankau, 1975b). Consequently, B. penetrans was never accepted for placement in Bergey’s Manual and as such the taxonomic position of this organism remained uncertain.

Sayre and Wergin (1977) examined the life cycle of the bacterial endoparasite of root-knot nematodes by electron microscopy and found it to have more in common with Actinomycetales and the bacterium P. ramosa than with members of the genus Bacillus. A detailed analysis of this bacterial parasite followed and the organism was renamed Pasteuria penetrans because of its similarity to P. ramosa (Sayre and Starr, 1985). P. penetrans was accepted for placement in the Bergey’s manual and is currently the accepted name.

Pasteuria Taxonomy

Pasteuria spp. are differentiated by host preference, developmental characteristics, and size and shape of sporangia and endospores (Sayre and Starr, 1989). Currently, five
species of *Pasteuria* have been described in detail. These are (i) *Pasteuria ramosa* which parasitizes water fleas of the genera *Daphnia* and *Moina* (type species of the genus), (ii) *P. penetrans* on *Meloidogyne* spp., (iii) *P. thornei* on *Pratylenchus* spp., (iv) *P. nishizawae* on cyst nematodes of the genera *Heterodera* and *Globodera* (Sayre and Starr, 1989, and (v) ‘*Candidatus Pasteuria usgae*’ from *Belonolaimus longicaudatus* in Florida (Giblin-Davies et al., 2003).

Several other *Pasteuria* isolates have been reported on various nematode hosts and in many different environments throughout the world (Atibalentja et al., 2000; Chen and Dickson, 1998). The genes encoding 16S ribosomal RNA have been sequenced for *P. ramosa* and for *Pasteuria* spp. isolated from root-knot nematodes (*M. arenaria* race 1 and *Meloidogyne* spp.), soybean cyst (*H. glycines*), and sting (*B. longicaudatus*) nematodes. The results obtained have provided a phylogenetic basis for their designation to a distinct clade within the family *Alicyclobacillaceae* (Preston et al., 2003).

Chen and Dickson (1998) have summarized the host and temperature preferences, pathogenicity, and occurrence of various *Pasteuria* isolates reported from all over the world. Endospores of *Pasteuria* spp. show remarkable size variations, ranging from approximately 2 to 8 μm. This wide range of host nematodes, endospore dimensions, and host specificity of *Pasteuria* spp. has caused considerable confusion in their taxonomy. Only five species of *Pasteuria* having been fully described that are associated with specific genera of plant-parasitic nematodes. Obviously there remains numerous *Pasteuria* yet to be named, e.g., *Pasteuria* specific to either ring or dagger nematodes. Some *Pasteuria* are able to parasitize nematodes across genera and it is unlikely that there will be a new species for each nematode genus (Bhattacharya and Swarup, 1988; Mankau
and Prasad, 1977; Oostendorp et al., 1990; Pan et al., 1993; Sharma and Davies, 1996; Vargas and Acosta, 1990).

**Life Cycle**

The life cycle of *P. penetrans* involves four stages: (i) endospore attachment to J2, (ii) germination of the endospore and penetration by germ tube of the cuticle, hypodermis and musculature, (iii) development within the infected nematode pseudocoel, and (iv) release of mature endospores. Endospores are the final developmental stage of the bacterium. They attach to the cuticle of J2 of *Meloidogyne* spp. when they move through the soil searching for a host plant. Germination of endospores occurs 4 to 10 days after the nematode enters a plant root and begins to feed (Sayre and Wergin, 1977; Serracin et al., 1997). After entering the nematode pseudocoelom, the germ tube develops into a cauliflower-like microcolony consisting of a dichotomously branched septate mycelium. Daughter colonies form when the intercalary cells in the microcolony lyse (Sayre and Starr, 1989). Due to unknown triggers, the colony fragments. The terminal cells of the fragments enlarge and undergo sporogenesis. Eventually, quartets and doublets of developing sporangia predominate in the nematode body cavity and finally separate into a single sporangium containing an endospore.

The mature endospores are released into the soil when the plant root with its complement of parasitized root-knot nematode female decomposes. It is not clear when the endospores are released in soil or when is the most opportune time to carry out a soil bioassay to accurately detect *P. penetrans* endospores in the soil.

**Systematics and Phylogeny**

Currently, endospore-forming bacteria are placed in 13 genera, which are based on morphological, physiological, and genetic diversity. The 16S rDNA sequences for
*Pasteuria* spp. were obtained from endospores of *P. ramosa* (Ebert et al., 1996), *P. penetrans* (Anderson et al., 1999), *Pasteuria* sp. from soybean cyst nematode (Atibalentja et al., 2000), and *Pasteuria* sp. from sting nematodes (Bekal et al., 2001). The results suggest that *Bacillus tusciae, Alicyclobacillus cycloheptanicus,* and *A. acidocaldarius* are the nearest relatives (Anderson et al., 1999; Ebert et al., 1996). The sequence analysis showed that *P. ramosa* does not belong to the Actinomycetales, as had been previously suggested (Bird, 1986; Sayre and Wergin, 1977).

The endospore morphology and sporogenesis of *P. penetrans* was similar to that of a typical bacterium, except that *P. penetrans* has a more complex polymorphic life cycle. Based on these characteristics, *Pasteuria* spp. belongs to members of the true bacteria (Chen et al., 1997; Ebert et al., 1996). Two apparent biotypes of *P. penetrans* demonstrating a host preference for different *Meloidogyne* spp. showed identical 16S rDNA sequences suggesting host recognition evolves within a given species (Preston et al., 2003). The sequence of genes encoding sporulation transcription factors *sigE* and *sigF* from *P. penetrans* biotype P-20 show different phylogenetic relationships to other endospore forming bacteria, supporting their application to further differentiate *Pasteuria* spp. and biotypes (Preston et al., 2003).

**Host Records**

Host records of *Pasteuria*-like organisms have been reviewed by Sayre and Starr (1988), Sturhan (1985), and Chen and Dickson (1998). *Pasteuria*-like organisms have been reported from 323 species of soilborne nematodes belonging to 116 genera, from 79 countries worldwide. The host nematodes include free-living, predacious, plant parasitic, and entomopathogenic nematodes.
Cultivation

Current methods of mass-producing *P. penetrans* rely on multiplication of the pathogen in its nematode host on greenhouse-grown plants (Stirling and Wachtel, 1980). The production systems might be improved by culturing the nematode and pathogen in excised or transformed root cultures (Verdejo and Jaffee, 1988; Verdejo and Mankau, 1986). Commercial use of the pathogen will most likely require an *in vitro* method of cultivation and various media have been tested for artificial cultivation of *Pasteuria* spp., but have been unsuccessful (Bishop and Ellar, 1991; Reise et al., 1988; Williams et al., 1989). Studies looking into the chemical and physiological changes in the pseudocoelom of the female nematodes as it matures may give clues as to the critical physiological requirements that may be required for the formulation of artificial medium for cultivation of *P. penetrans*.

Effects of Temperature, Moisture, and pH on *P. penetrans*

The development of *P. penetrans* in nematodes is temperature dependent (Freitas et al., 1997; Hatz and Dickson, 1992; Serracin et al., 1997; Stirling, 1981; Talavera and Mizukubo, 2003). Development of *P. penetrans* within females of *M. javanica* and *M. arenaria* was not observed at 10 °C (Hatz and Dickson, 1992). The minimal developmental temperature was determined as 17 °C (Chen and Dickson, 1997a), with optimal growth temperature between 28 °C and 35 °C (Hatz and Dickson, 1992; Serracin et al., 1997). Based on these temperature requirements, *P. penetrans* can be defined as a mesophilic bacterium. However, different temperature requirements exist for various isolates of the bacterium due to its cosmopolitan distribution. An Indian isolate of *Pasteuria* spp. that infects both *Heterodera* spp. and *M. incognita* completed its life cycle in *M. incognita* in 49 days at 10 °C to 17 °C (Bhattacharya and Swarup, 1988).

There is little knowledge on the effect of soil moisture on endospore attachment and development of *P. penetrans*. It has been reported that the proportion of J2 with attached endospores was greater in moistened soil than in dry soil (Brown and Smart, 1984). The rate of development of *P. penetrans* in infected females was reduced when soils were maintained at or near field capacity (Davies et al., 1991). Although the reasons for these effects are not known, it is possible that oxygen depletion in wet soil inhibits respiration, resulting in an inhibition of development of both the nematode and bacterial parasite.

Endospore attachment is affected by pH. The highest attachment occurred at pH 9 (Ahmed and Gowen, 1991) and decreased at low pH values (Ahmed, 1990). Davies et al. (1988) observed that the attachment was higher at pH 7 than pH 4 or pH 9 in tap water, but lower at pH 7 than pH 4 or pH 9 in distilled water and tap water. Attachment of sonicated endospores was higher at pH 7 than pH 4 or pH 9 in distilled water and tap water. Sonicated endospores attached in higher numbers per J2 in tap water than in distilled water (Davies et al., 1988). Studies have revealed that the endospore surface has a net negative charge, which was greater at neutral pH and was reduced with a change of pH away from neutral (Afolabi et al., 1995). Electrostatic forces between the nematode cuticle and the endospore surface oppose attachment because the charges on nematode
cuticle were also negative. Reasons for the pH effects are still unclear (Afolabi et al., 1995).

**Fate of Endospore in the Soil**

The long-term survival of endospore of *P. penetrans* in soil has not been well defined. In a peanut field in Florida, *P. penetrans* endospores maintained suppressive levels for *M. arenaria* over 10 years (Dickson et al., 1994). Endopores of *P. penetrans* resist various chemicals and environmental conditions (Mani, 1988; Williams et al., 1989). In a laboratory study, endospores of *Pasteuria* sp. were reported viable for a period of more than 1 year (Mani, 1988). Storage of endospores for 5 years and 11 years did not affect their rate of attachment, but decreased their rate of infection (Giannakou et al., 1997). Endospores of *P. penetrans* were killed by autoclaving but only slightly affected by microwaving (Weibelzahl-Fulton, 1998).

Natural enemies of *P. penetrans* endospores in soil have not been reported. A bacterium has been observed attached to endospores when they were attached to the cuticle of J2 of *Meloidogyne* spp. (Chen and Dickson, 1998). The bacterium is rod-shaped, gram-negative and may not be a parasite of the endospore because the ultrastructure and morphology of endospores remained intact when the bacteria were present.

**Biological Control Potential**

*Pasteuria penetrans* has been shown to effectively suppress root-knot nematode populations in field and microplots experiments (Brown and Smart, 1985; Chen and Dickson, 1998; Chen et al., 1996b; Daudi et al., 1990; Dickson et al., 1994; Freitas et al., 2000; Oostendorp et al., 1991; Stirling 1984; Trivino and Gowen, 1996; Weibelzahl-Fulton et al., 1996). The role of *P. penetrans* in suppressing plant-parasitic nematodes
has been tested on many crops, mostly in greenhouse pots (Chen and Dickson 1998). *P. penetrans* suppressed *Meloidogyne* spp. on egg-plant, tomato, wheat, tobacco, soybean, bean, pepper, hairy vetch, cucumber, peanut, rye, chickpea, kiwi, grape, brinjal, mung, and okra. Isolates of *Pasteuria* spp. have been reported to suppress *Heterodera avenae* and *H. zeae* on bermudagrass turf (Giblin-Davis, 1990), *H. elachista* on rice (Nishizawa, 1987), and *H. cajani* on cowpea (Singh and Dhawan, 1994).

Cross-genera suppression of nematodes was also observed. Mankau and Prasad (1972) reported that *P. penetrans* reduced tomato root galls induced by *M. javanica* and *M. incognita*. *Pratylenchus scribneri* was reduced by 53% in soil and 63% in roots 55 days after the nematodes were inoculated in *P. penetrans* infested soil (Mankau and Prasad, 1972). An Indian isolate of *P. penetrans* parasitized both *Heterodera* spp. and *M. incognita* (Bhattacharya and Swarup, 1988). The bacterium inoculum was mass-produced on *M. incognita* and when the inoculum was mixed into soil, the numbers of *H. avenae* cysts on wheat were reduced.

**Nematode Suppressive soils**

**Definition**

Suppressive soils are defined as those ecosystems in which a population increase of a plant-parasitic nematode is much lower than in a conducive soil despite the presence of a susceptible host, a virulent pathogen, and suitable environmental conditions (Stirling, 1991).

**Historical Background**

There are only a few documented reports of plant-nematode-suppressive soils, with most regarding fungal antagonists (Gair et al., 1969; Jaffee and Zehr, 1982; Kerry 1982; Stirling et al., 1979). During the past 26 years there have been more reports regarding
suppressive soils infested with large numbers of *P. penetrans* (Bird and Brisbane, 1988; Dickson et al., 1991; 1994; Mankau, 1980; Minton and Sayre, 1989; Stirling and White, 1982). Nematode-suppressive soils are often first recognized or suspected when population densities of the nematode decline after initial establishment (Gair et al., 1969) or when populations remain significantly lower in some fields than in other fields in the same area with similar soil and crop histories (Carris et al., 1989; Westphal and Becker, 1999). Baker and Cook (1974) defined soil suppressiveness for soilborne disease as “the inhostipality of certain soils to some plant pathogens such that either the pathogen cannot establish, they establish but fail to produce disease, or they establish and cause disease at first but diminish with continued culture of the crop”. Nematode-suppressive soils occur widely, but only a limited number of examples have been demonstrated to be biological in nature (Crump, 1989; Kerry, 1988). Suppressive soils often are associated with monoculture of a susceptible host (Gair et al., 1969; Hartwig, 1981; Heijbroek, 1983; Noel and Wax, 2003; Timper et al., 2001; Westphal and Becker, 1999). Monoculture does not necessarily lead to a nematode-suppressive soil (Carries et al., 1989).

Soils with specific suppressiveness to plant-parasitic nematodes are of interest to define the mechanisms that regulate population density (Westphal, 2005). Suppressive soils prevent nematodes from causing disease and they diminish disease severity after initial nematode damage in continuous culturing of a host (Westphal, 2005). Field observations of suspected nematode-suppressive soils must be confirmed by greenhouse tests to determine the intrinsic character of the soil suppressiveness (Westphal, 2005).
General soil suppressiveness is distinguished from specific suppressiveness by the fact that the latter is transferable (Westphal, 2005). The biological nature of specific suppressiveness is confirmed when suppressiveness is (i) eliminated by biocidal treatments, (ii) transferred to conducive soils with small portions of suppressive soil, (iii) specific to a particular pathogen (Kerry, 1988), (iv) observed as reduced reproduction in cyst and root-knot nematodes in the root zone, (v) isolated by baiting techniques, (vi) heat sensitive, and (vii) density dependent (Westphal and Becker, 2001a,b,c).

Some fungi and bacteria, such as certain *Fusarium* spp., *Verticillium* spp., and *P. penetrans*, have host ranges that include both cyst and (or) several root-knot nematodes (Davies et al., 2001; Godoy et al., 1983; Meyer et al., 1990; Qadri and Saleh, 1990). Soil suppressiveness against *H. schactii* was shown also to be effective against *M. incognita* (Pyrowolaskis et al., 2002). In these tests, soil suppressiveness was eliminated by soil fumigation. Such tests are valuable for characterizing soil suppressiveness in greater detail because components of soil suppressiveness that are active against particular life stages of a nematode may impact similar life stages of other nematodes. For *P. penetrans* induced suppressiveness, it is important to test for infection rather than attachment alone because some *Pasteuria* spp. can attach to nematode cuticles but not infect (Carneiro et al., 2004; Davies et al., 1990). Endospores attaching to the nematode cuticle but not infecting the nematode are likely to be of limited value as biocontrol organisms (Westphal, 2005).

Exploitation of soil suppressiveness for nematode management has been difficult because such soils are often induced under non-practical monoculture with susceptible host plants and the resulting initially high population densities of the plant parasites
(Westphal, 2005). Recent evidence suggests that suppressiveness can also develop under resistant host plants (Noel and Wax, 2003).

**Transferability of Suppressiveness**

Transferability of suppressiveness is an indication of specific soil suppressiveness against plant-parasitic nematodes (Kerry, 1988), particularly when the nematode antagonists are not culturable or are unknown. In soilborne diseases, specific suppressiveness is transferable to conducive soils with small portions of soil. This observation is considered an indicator of the biological nature of suppressiveness (Menzies, 1959; Shipton et al., 1973).

Amendment of steam-sterilized greenhouse soil with *P. penetrans* infested soil resulted in suppression of *M. incognita* (Mankau, 1975a). Transfer of the 20 to 53-μm fraction of a soil derived from northern Europe that contained *Nematophthora gynophila* to South Australia soils infested with *Heterodera avenae* resulted in fungal infection of the nematode (Stirling and Kerry, 1983). A soil suppressive site to *M. arenaria* race 1 has been reported that has been linked to *P. penetrans* isolate P20 (Dickson et al., 1994), but there are no studies done so far to test whether this suppressiveness can be transferred from one site to another site.

**Effect of Chemicals Nematicides on *P. penetrans* Suppressiveness**

The use of *P. penetrans* as a biological control agent in combination with other management practices, especially nematicides, is of interest (Freitas, 1997). Stirling (1984) reported that infection of *M. javanica* by *P. penetrans* after treatment *in vitro* was not affected by nematicides 1,3-D or DBCP. Brown and Nordmeyer (1985) demonstrated a synergistic reduction of root galling by *M. javanica* with carbofuran or aldicarb combined with *P. penetrans*. A possible explanation for the synergism was that
low concentrations of the carbamate nematicide stimulated nematode movement and orientation toward host roots. Thus, the probability of nematode contact with bacterial endospores was increased. Organophosphates and carbamate nematicides at high concentrations have been shown to decrease nematode mobility, thereby the most likely explanation of reduced infection was the decreased probability of contact between nematodes and endospores.

Chloropicrin and methyl bromide + 33% chloropicrin were highly detrimental to *P. penetrans* in field and greenhouse experiments. Treatments with 1,3-D + 17% chloropicrin, 1,3-D + 25% chloropicrin, and 1,3-D + 35% chloropicrin had moderate effects on the bacterium. However metam sodium did not have a deleterious effect on the bacterium (Freitas, 1997). Freitas (1997) postulated that the chloropicrin present in these formulations may have been responsible for the bactericidal effect on *P. penetrans*. Further field studies are necessary to determine whether other pesticides are detrimental to *P. penetrans*.

**Effect of Cropping System and Nematode Density on *Pasteuria penetrans* Supressiveness**

Abundance of *P. penetrans* endospores has been reported highest in peanut monoculture and intermediate in two rotations of bahiagrass and one rotation of cotton (Timper et al., 2001). Cetintas and Dickson (2004), while studying the long-term persistence and suppressiveness of *P. penetrans* against *M. arenaria* race 1, reported the percentage of J2 with endospores was highest in weed fallow (87%) followed by bahiagrass (63%) and rhizomal peanut (53%).

Oostendorp et al. (1990) established a field microplot experiment to determine whether the cropping sequence of peanut and different winter cover crops influenced
infection of *M. arenaria* by *P. penetrans* and improved peanut growth over time. They concluded that the number of plots with *P. penetrans* infection was higher in rye and vetch plots than in fallow plots and that the number of *P. penetrans* spores adhering to J2 in soil increased continuously over 3-years and was influenced by the cropping sequence. Differences in numbers of *M. arenaria* in plots without *P. penetrans* among three cropping sequences were observed only in the spring of each year and not in autumn. This suggested that the summer crop, which was peanut, had a stronger influence on the nematode population density than did the winter cover crops.

It has been demonstrated that when *P. penetrans* is introduced into a soil containing high densities of *M. arenaria*, the bacterium will amplify to suppressive levels within 3 years (Oostendorp et al., 1990, 1991) or sooner if higher densities of endospores (100,000/g of soil) are added (Chen et al., 1996b). Peanut may be an ideal crop for amplifying *P. penetrans* to suppressive densities because it is grown in a hot climate and is a relatively long season crop. Both of these conditions favor development of *P. penetrans* (Hatz and Dickson, 1992; Serracin et al., 1997). Also, methods for harvesting the peanut crop that include digging plants, drying on the soil surface, and then combining pods, leave behind root residues which most probably aid in the spread of endospores (Dickson and De Waele, 2005). Over a period of 3 to 5 years, peanut pods and pegs may be totally free of visible galls where *P. penetrans* occurs. With reduction of J2 densities in the soil, *P. penetrans* density may also diminish.

In order to maintain soil suppressiveness due to *P. penetrans*, some amplification of the bacterium in the soil must occur (Cetintas and Dickson, 2005). The downward dispersal of endospores with percolating water could result in depletion of *P. penetrans*
endospores from the top 20 to 25 cm of the soil if they are not being continuously amplified in this zone (Cetintas and Dickson, 2005). This may require that nematode population densities be maintained at low levels to maintain suppressiveness (Cetintas and Dickson, 2005). Additional long-term experiments are needed to determine whether nematode population densities can be maintained at acceptable low levels and at the same time continually amplify *P. penetrans*. The influence of different summer crops and different densities of root-knot nematode juveniles on the abundance and amplification of *P. penetrans* need to be evaluated.

Objectives

The objectives of the research reported herein were (i) to study the effect of endospore density on attachment, penetration, and production of egg masses in infected root-knot nematode females, (ii) to transfer *P. penetrans* induced suppressiveness from one site to another site where a similar suppressiveness has not been reported and to evaluate the suppressiveness using two soil fumigants, and (iii) to study the effect of different summer crops and nematode levels on *P. penetrans* amplification.
CHAPTER 2
EFFECT OF *Pasteuria penetrans* ENDOSPORE DENSITY ON ATTACHMENT, PENETRATION, AND FECUNDITY OF *Meloidogyne arenaria* RACE 1

Introduction

*Pasteuria penetrans* (Thorne) Sayre & Starr is an obligate, mycelial endospore forming bacterial parasite of root-knot nematode that has shown potential as a biological control agent of plant-parasitic nematodes in microplot, greenhouse, and field experiments (Chen et al., 1996b; Dickson et al., 1994; Oostendorp et al., 1991; Tzortzakakis et al., 1997; Trudgill et al., 2000). Attachment is the first step toward successful development of *P. penetrans* within its nematode host (Afolabi et al., 1995; Davies et al., 1996; Talavera and Mizukubo, 2003). Percentage number of J2 with endospores attached and the number of endospores attached per J2 is reported to be influenced by concentrations of endospores in soil as well as soil texture and temperature (Ahmed and Gowen, 1991; Freitas et al., 1997; Hatz and Dickson, 1992; Nakasono et al., 1993; Rao et al., 1997; Serracin et al., 1997; Stirling, 1981; Stirling, 1984; Stirling et al., 1979, 1990; Talavera and Mizukubo, 2003).

Endospores of *P. penetrans* are nonmotile, thus for them to come in contact with nematodes in the soil depends on nematode movement (Stirling et al., 1990; Talavera and Mizukubo, 2003). It is likely that nematode mobility in the soil profile is the single most important factor favoring attachment of endospores to the nematode cuticle. The more active nematodes have a greater chance of coming in contact with endospores. However,
it is known that endospores readily move downward in the soil profile with percolation of water (Cetintas and Dickson, 2005; Oostendorp et al., 1989).

The percentage of J2 with endospores attached and number of endospores per J2 have been correlated with infection in adult nematodes and therefore is often used as an indirect measure of biocontrol potential or as a method to estimate endospore concentrations in the soil (Chen and Dickson, 1997b; Dabire et al., 2001; Rao et al., 1997; Stirling, 1984). However, mere attachment does not mean that endospores will germinate and enter the nematode body. For *P. penetrans* to be a successful parasite it must attach and then penetrate. It is only after penetration and development within the nematode pseudocoelom that one can conclude the bacterium is a successful parasite.

It has been reported that endospore attachment hinders nematode mobility and infectivity, and if the bacterium ultimately infects and develops within the nematode host it affects nematode fecundity (Ahmed and Gowen, 1991, Davies et al., 1988; Stirling, 1984; Stirling et al., 1990). These data were generated in field and greenhouse trials in diverse pathosystems and with varied results. To better understand the effect of endospore numbers on infectivity and fecundity of nematode hosts, trials conducted under more environmentally controlled conditions should be done. The objectives were to determine the effects of different *P. penetrans* endospore concentrations on (i) attachment, (ii) nematode infectivity in plant roots, (iii) galling, and (iv) egg mass production as a measure of fecundity.

**Materials and Methods**

**Nematode Source**

The isolate of *M. arenaria* race 1 originated from peanut grown at the former University of Florida Green Acres Agronomy Farm, Alachua County, FL, and was
maintained in a greenhouse on tomato (*Lycopersicum esculentum* Mill cv. Rutgers). The speciation and race designation was confirmed by examination of morphometrics, isozyme phenotypes, and host differentials. Eggs from infected tomato roots were extracted using a 0.5% sodium hypochlorite solution (Hussey and Barker, 1973). For the multiplication of *P. penetrans, M. arenaria* inoculum was obtained by hatching J2 from eggs over a 48-hour period after discarding J2 emerging during the first 24 hours.

**Bacterial Culture**

An isolate of *P. penetrans* designated P-20 that originated from *M. arenaria* race 1 collected from peanut in Levy County, FL was used (Oostendorp et al., 1990). Endospores were obtained from infected female nematodes and centrifuged to improve their attachment level to the cuticle of 1 to 3 day old *M. arenaria* J2 (Hewlett and Dickson, 1993). Tomato plants (45-day-old seedlings) grown in 15-cm-diam. clay pots were inoculated with 3,000 endospore encumbered J2/pot. Three days later the plants were again inoculated and maintained in a greenhouse at 27 ± 5 °C. They were fertilized twice weekly by adding 50 ml of a solution containing 0.63 g/liter of 20-20-20 (N-P-K), watered daily, and insecticides and fungicides applied as needed.

Sixty days after inoculation the roots were harvested, washed with tap water, and incubated for 2 to 3 days at room temperature in 10% Rapidase Pomaliq 2F (Gist-Brocades Pomaliq product number 7003-A/DSM, Food Specialties USA, Menominee, WI), 50 mM NaAcetate (pH 5.0), and 0.1% CaCl₂ at ca. 50:50 v/v (Charnecki, 1997). The roots were digested for 3 days and then placed on a 600-µm-pore sieve nested on a 150-µm-pore sieve and females dislodged by subjecting them to a high pressure spray of water. Endospore-filled females were hand picked with forceps under a dissecting microscope at 200× magnification and placed in 1.5-ml siliconized microtubes that
contained 1-ml deionized water. They were washed with deionized water (dH2O) before rupturing using a smooth mortal and pestle and the contents passed through a woven 21-μm opening polyester filter in a 13-mm Swinnex disc holder (Fisher Scientific, Suwanee, GA). Recovered endospores were washed twice in distilled water through centrifugation at 10,000 g for 5 minutes, and stored at 4 °C. One milliliter of the endospore solution was placed on a hemocytometer and the number of endospores per milliliter estimated by counting at 400× magnification with a microscope.

Attachment Experiment

Freshly harvested *P. penetrans* endospores were used. Endospore concentrations were adjusted to obtain the following J2-to-endospore ratios; 1:50 (1 J2 for every 50 endospores), 1:100, 1:150, and 0 endospores for the control, each in a volume of 2.5-ml deionized water and then agitated thoroughly. The mixture was centrifuged at 5,500 g for 5 minutes (Hewlett and Dickson, 1993). Each treatment had 15,000 J2 added. A 2-ml suspension was placed on a counting slide and observed for endospore attachment with a microscope at 400× magnification. The first 20 J2 in each treatment were observed for number of *P. penetrans* endospores attached to the cuticle with a microscope at 400× magnification.

Penetration

This experiment was conducted in an enviromental chamber maintained at 27 °C, 60% relative humidity (RH), and 14-hours of light for 7 days (Walker et al., 1993). The treatments were the four attachments levels as mentioned above. Tomato cv. Rutgers seedlings growing in 8-cm-diam. pots containing autoclaved sand were inoculated with 500 J2/plant (<48 hours old) from each of the attachment levels. The treatments were
arranged in a randomized complete block design, and replicated four times. The experiment was repeated.

**Root Clearing**

Seven days after inoculation the tomato plants were harvested by gently removing the roots from the cups. The roots were washed, cleared, and stained by a root clearing technique (Byrd et al., 1983). Individual root pieces were pressed between two glass microscope slides and the number of J2 that had penetrated the roots were counted with a microscope at 200× magnification.

**Gall and Egg Mass Production**

The experiment was conducted in a greenhouse where temperatures averaged 27 ± 5 ºC. The same treatments as applied for the penetration experiment were used to inoculate 45-day-old tomato seedlings (cv. Rutgers) growing in 15-cm-diam. clay pots. Each plant was inoculated with 3,000 J2 with endospores attached as described for the previous experiment above. Plants were watered, insecticides and fungicides applied as needed, and fertilized weekly with a solution containing 0.63 g/liter of 20-20-20 (N-P-K). The four treatments were arranged in a randomized complete block design replicated four times. Thirty days after inoculation, the roots were harvested and the number of galls and egg masses were rated using a 0 to 5 scale where; 0 = no galls or egg masses, 1 = 1-2 galls or egg masses, 2 = 3-10 galls or egg masses, 3 = 11-30 galls or egg masses, 4 = 31-100 galls or egg masses, 5 = >100 galls or egg masses (Taylor and Sasser, 1978). To aid in the visualization of egg masses the roots were washed carefully, blotted dry, and placed in a 500 ml beaker containing 20% solution of red food coloring for 15 minutes, after which the roots were rinsed in tap water and blotted dry (Thies et al., 2002).
Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the GLM procedure in SAS/STAT version 9.1 (SAS Institute, Cary, NC). Means were separated and compared using Waller-Duncan $k$ ratio $t$-test and reported as significant at the 95% confidence level. Endospores per J2 data were transformed using $\log_{10} (x+1)$ before statistical analysis. Untransformed numbers are presented in the text.

Results

Attachment

Average attachment levels of $3.5 \pm 0.4$, $10.2 \pm 1.0$, and $18.1 \pm 2.1$ endospores/J2 were obtained from J2-endospore ratios of 1:50, 1:100, and 1:150, respectively. All these attachment levels were different ($P \leq 0.05$) (Table 2-1). No attachment of endospores was observed in the control.

Penetration, Galling and Egg Masses

The number of J2 penetrating roots was reduced by an increasing number of endospores attached to their cuticle ($P \leq 0.05$) (Table 2-2). When the mean attachment level was 0, $148 \pm 9.0$ J2 were found penetrating roots. When the mean endospore attachment was 3.5, $68.8 \pm 3.9$ J2 were found penetrating, whereas when the mean attachment levels were 10.2 and 18.1, the number of J2 found penetrating roots was $30.4 \pm 1.6$ and $28.2 \pm 2.7$, respectively (Table 2-2). There were no differences between the two higher attachment levels in J2 penetration or galling but egg mass production was reduced at the higher attachment level ($P \leq 0.05$). The attachment levels and the number of J2 penetrating per root system were negatively correlated but the regression equation was not significant ($P \leq 0.05$). Galling and egg mass indices were greater for the control
than that for all other treatments ($P \leq 0.05$). The higher attachment levels greatly reduced egg masses ($P \leq 0.05$).

Discussion

The number of endospores attached per J2 plays an important role in root penetration and nematode fecundity. It has been reported that the number of endospores attached to the cuticle of J2 increased in proportion to both endospore concentration and time (Stirling et al., 1990). The effect of endospore concentrations in soil environments is important in understanding this organism’s role in causing soil suppressiveness. Although attachment does not necessarily imply infection (Carneiro et al., 2004), it is obviously an important step in the infection process (Talavera and Mizukubo, 2003). Studies have shown that attachment is important in inferring host specificity of *P. penetrans* isolates (Brito et al., 2003; Davies et al., 1992; Oostendorp et al., 1990).

As the mean attachment level per juvenile increases, the percentage of J2 penetrating roots decreases. The number of J2 entering plant host root were reported to be reduced when they were encumbered with 15 or more endospores (Davies et al., 1988), whereas others reported that 11 or more endospores/J2 reduces their ability to enter host roots (Ahmed and Gowen, 1991). Even higher numbers of 25 to 30 endospores/J2 have been reported preventing root penetration (Stirling, 1984; Stirling et al., 1990). In this study as few as 3.5 endospore/J2 reduced the ability of *M. arenaria* to enter host roots. This supports the hypothesis that one way *P. penetrans* induces soil suppressiveness is by interfering with root penetration (Stirling, 1984). Reduced root penetration may most likely be attributed to reduced mobility of endospore encumbered J2 (Sturhan, 1985). Generally, the higher the endospore attachment levels the lower were the rates of penetration; however, linear and exponential equations to explain the
relationship were not significant. The low levels of penetration (30% in control) in both experiments could be attributed to the fact not all the J2 had penetrated the roots by day 7 when the experiment was terminated.

Root galling was affected by levels of endospore attachment. There were differences between the control and all other treatments. Once the endospore encumbered J2 enters plant roots, apparently the bacterium begins the infection process by inserting a germ tube through the nematode cuticle-hypodermis to reach the pseudocoelom (Sayre and Starr, 1988), however the infected J2 is able to begin the formation of giant cells and galling results, but egg production is completely blocked or greatly reduced (Gowen et al., 1989). This study agrees with these findings. Gall formation is the host reaction to nematode infection and is independent of *P. penetrans* infection of the nematode. It is only following subsequent cropping sequences and build up of suppressiveness that the nematode population in the soil is reduced and the lack of galling can be used as an indicator of *P. penetrans* suppressiveness. On the contrary, egg mass production serves as a good indicator of *P. penetrans* suppressiveness. The study demonstrates that *P. penetrans* induced suppressiveness is manifested in the form of limited penetration and egg mass production (fecundity).
Table 2-1. Effect of endospore:second-stage juvenile (J2) ratios on attachment of *Pasteuria penetrans* endospores to *Meloidogyne arenaria* race 1.

<table>
<thead>
<tr>
<th>J2:endospore ratios</th>
<th>Mean attachment (Endospore/J2&lt;sup&gt;a&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50</td>
<td>3.5 ± 0.4 c</td>
</tr>
<tr>
<td>1:100</td>
<td>10.2 ± 1.0 b</td>
</tr>
<tr>
<td>1:150</td>
<td>18.1 ± 2.1 a</td>
</tr>
</tbody>
</table>

Means within each column followed by the same letter are not significantly different according to Waller-Duncan k ratio t-test ($P \leq 0.05$).

<sup>a</sup>Each value is a mean of four replicates. For each replicate 20 J2 were observed for the number of endospores that were attached to the nematode cuticle.
Table 2-2. Effect of different attachment levels of endospores of *Pasteuria penetrans* to second-stage juveniles of *Melodogyne arenaria* race 1 on root penetration, gall formation, and egg mass production.

<table>
<thead>
<tr>
<th>Attachment levels</th>
<th>J2 penetrating&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Gall index&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Egg mass index&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>148.0 ± 9.0 a</td>
<td>5.0 ± 0.1 a</td>
<td>4.8 ± 0.2 a</td>
</tr>
<tr>
<td>3.5</td>
<td>68.8 ± 3.9 b</td>
<td>4.5 ± 0.2 b</td>
<td>4.0 ± 0.3 b</td>
</tr>
<tr>
<td>10.2</td>
<td>30.4 ± 1.6 c</td>
<td>4.0 ± 0.1 c</td>
<td>2.8 ± 0.3 c</td>
</tr>
<tr>
<td>18.1</td>
<td>28.2 ± 2.7 c</td>
<td>4.0 ± 0.1 c</td>
<td>1.5 ± 0.2 d</td>
</tr>
</tbody>
</table>

Means within each column with the same letter are not significantly different according to Waller-Duncan $k$ ratio $t$-test ($P \leq 0.05$).

<sup>a</sup>Each value is a mean of four replicates.

<sup>b</sup>Galling and egg mass indices were rated using a 0 to 5 scale where; 0 = no galls or egg masses, 1 = 1-2 galls or egg masses, 2 = 3-10 galls or egg masses, 3 = 11-30 galls or egg masses, 4 = 31-100 galls or egg masses, 5 = >100 galls or egg masses (Taylor and Sasser, 1978).
CHAPTER 3
TRANSFER AND MANAGEMENT OF *Pasteuria penetrans* AND *Meloidogyne arenaria* RACE 1

Introduction

The peanut root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood race 1 is a widespread and destructive nematode pathogen of peanut (Minton and Baujard, 1990; Porter et al., 1984). The nematode infests 25% of the peanut acreage in the southeastern United States (Bridges et al., 1994). It is a dominant, aggressive pathogen of peanut and causes substantial yield losses at relatively low population densities (McSorley et al., 1992; Wheeler and Starr, 1987). In addition to the loss of quality and quantity of peanut yield, nematode damaged seed kernels of peanut are susceptible to infection by *Aspergillus flavus*, *A. parasiticus* and other fungal diseases prior to harvest (Abawi and Chen, 1998; Cole et al., 1995; Sanders et al., 1993; Timper et al., 2004). After colonization of the kernel, these fungi can produce toxic metabolites known as aflatoxins under certain conditions. Aflatoxins are potent carcinogens and are a major concern for the peanut industry (Lamp and Sternitzke, 2001).

The current approach to the management of the peanut root-knot nematode include the use of chemicals nematicides (both fumigants and nonfumigants), cultural practices, and resistant cultivars (Dickson and De Waele, 2005; Kinloch, 2003; Rich and Kinloch, 2005). All of these may be used in an integrated manner.

There has been an increased interest in developing biological control agents as an alternative for nematode management in peanut production. Currently, however, none
exist that can be sufficiently mass produced for introducing into root-knot nematode infested peanut fields. There are only a few examples of effective biological control of plant nematodes, and methodologies for demonstrating effectiveness of these agents are still under development. However, there have been a few biocontrol agents demonstrated as playing an important role in soil suppressiveness (Chen and Dickson, 1998; Dickson et al., 1994; Kerry 1982). Of all the biological agents studied Pasteuria penetrans (Thorne) Sayre & Starr has perhaps shown the greatest potential for biocontrol of root-knot nematodes, especially against root-knot disease of peanut (Dickson et al., 1994; Chen and Dickson, 1998). *P. penetrans* is an endospore-forming, gram-positive bacterial parasite specific to species within the *Meloidogyne* spp. and has been demonstrated to be capable of causing soil suppressiveness to these nematodes (Brown and Smart, 1985; Chen et al., 1996b; Freitas et al., 2000; Oostendorp et al., 1991; Stirling 1984; Weibelzahl-Fulton et al., 1996).

No studies have been carried out to determine whether the *P. penetrans* can be transferred, established, and managed in a new field site. The objective of the current study was to investigate whether *P. penetrans* can be transferred from a suppressive site to a new site and to investigate the effect of two soil fumigants, 1,3-D and chloropicrin, on the development of *P. penetrans* at this new site.

**Materials and Methods**

**Transfer and Establishment of Pasteuria penetrans**

**Field site.** A 25 m × 185 m field located at the Plant Science Research and Extension Center, Citra, FL was prepared for infestation with *M. arenaria* and *P. penetrans*. The field was deep ploughed and disked before inocula were added. The soil was classified as Arredondo fine sand (92.7% sand, 3.9% silt, 3.4% clay and <1%
organic matter; pH 5.5). The site was chosen because no root-knot nematodes or Pasteuria spp. were detected in the soil.

**Bacteria source.** Pasteuria penetrans isolate P-20 (Oostendorp et al., 1990) was collected twice from a peanut field in Levy, County, FL that was known to be heavily infested with *M. arenaria* and *P. penetrans* (Cetintas and Dickson, 2004, 2005; Dickson et al., 1994). Peanut had been grown in the field for the past 4 years. In the autumn of 2002, the peanut crop was dug and left on the soil surface to dry for 2 weeks. The dried crop roots, pegs and pods were collected and spread by hand over the new field site. The dried material was incorporated into the soil ca. 10 cm deep with a rototiller. The Levy Co. field site was planted with okra (*Abelmoschus esculentus* L. Moench) cv. Clemson Spinless on 5 July 2003. The crop was uprooted with a peanut digger mid-September and left on the soil surface to field dry for 2 weeks. On 30 September 2003, the roots were collected and spread over the new field site by hand. The dried plant roots were incorporated into the soil ca. 10 cm deep with a rototiller.

**Nematode source.** Meloidogyne arenaria race 1 was introduced at the new field site on 21 March and again on 18 September 2003. The field was tracked on 1.8 m centers and a cultivating sweep was used to open furrows 20-cm deep in the center of the wheel tracks. The first introduction of *M. arenaria* was from infested soil and galled tomato roots. This inoculum, which was equally distributed in the open furrows and covered, was grown on tomato (*Lycopersicum esculentum* Mill. cv. Rutgers) in 500 15-cm-diam. pots. The inoculum for the second introduction was *M. arenaria* heavily galled peanut roots, pods and pegs obtained from a grower field in Levy Co., FL. Before infestation of the nematode in the new site, the identification of the nematode was
confirmed as *M. arenaria* based on morphology and isozyme phenotypes of females extracted from roots pegs and pods (Esbenshade and Triantaphyllou, 1985). No *P. penetrans* endospores were detected from females extracted from roots or J2 extracted from soil.

**Amplification of Pasteuria penetrans**

Peanut (*Arachis hypogaea* L. cv. Georgia Green) was planted 16 April 2003, followed by winter wheat (*Triticum aestivum* L. cv. AGS 2000) on 2 October 2003. The field was arranged into 54 plots, each consisting of four rows 15-m long by 91-cm wide with 91-cm row spacing. Production practices for growing peanut and wheat in the area were applied (Whitty, 2002; Mask et al., 1992). The peanut and wheat crops were ploughed under on 9 September 2003 and 21 April 2004, respectively. Soil samples were collected from each plot. Six cores (2.5-cm-diam., 20 cm deep) of soil were taken per plot with a cone-shaped sampling tube before planting and at harvest. The soil from each plot was mixed thoroughly, and nematodes were extracted from 100 cm$^3$ of soil by centrifugal-flotation method (Jenkins, 1964). The first 20 J2 were scored for presence or absence of endospores. Six plants were chosen randomly from each plot at harvest to determine root-knot nematode galling based on a scale of 0 to 5 (Taylor and Sasser, 1978).

**Soil Bioassay for Detection of Pasteuria penetrans in Soil**

A soil bioassay was conducted preplant and at harvest from each plot to determine the presence of *P. penetrans* in soil. Soil taken from each plot was air-dried and 40 g was placed in a 50-ml sterile polyethylene centrifuge tube. Soil water content was adjusted to 100% (saturated) capacity to increase the rate of endospore attachment to J2 (Brown and Smart, 1984). Then 500, 1-to 3-day-old J2 of *M. arenaria* were added and the tubes were
left uncovered at room temperature. Three days later, the J2 were extracted from the soil using the centrifugal-flotation method (Jenkins, 1964). Observations for the number of endospores attached per J2 were made with an inverted microscope at 200× magnification.

**Determining the Females of *Meloidogyne arenaria* Infected with *Pasteuria penetrans***

Galled roots from six peanut plants were collected randomly from each plot at harvest. Fresh roots, pods and pegs from each sample were incubated for 2 to 3 days at room temperature in 10% Rapidase Pomaliq 2F (Gist-Brocades Pomaliq product number 7003-A/DSM, Food Specialties USA, Menominee, WI), 50 mM NaAcetate (pH 5.0), and 0.1% $\text{CaCl}_2$ at ca. 50:50 v/v (Charnecki, 1997). Twenty milliliters of the solution was used per 4 g of root, pod, and peg mixture. The samples were washed vigorously over stacked-sieves with 600-µm (30 mesh) nested over a sieve with 150-µm-pore openings (100 mesh) and sprayed with a heavy stream of tap water (Hussey, 1971). Thirty females were randomly selected and crushed on a glass slide, covered with a cover slip, and examined under 400× for the presence or absence of *P. penetrans* endospores.

**Effect of Soil Fumigants on Development of *P. penetrans***

In the spring 2004, following the wheat crop, the field was arranged in a randomized complete block design with three treatments replicated 18 times. Each plot measured 6.1 m long × 3.6 m wide, with a 1.8 m wide non-tilled border separating each plot. The three treatments were: (i) nontreated control (*P. penetrans* and *M. arenaria* race 1 infested plots), (ii) chloropicrin at the rate of 263 kg/ha, and (iii) 1,3-D at the rate of 168 liters/ha. The fumigants were applied broadcast with six chisels each spaced 30 cm apart and were adjusted to deliver the fumigants 20 cm deep beneath the final soil
surface. The soil was compacted using a heavy roller to provide a surface seal to prevent premature loss of the fumigants to the atmosphere.

Two weeks following fumigation, peanut cv. Georgia Green was seeded in all plots. In summer 2004, due to the effect of two hurricanes, six replicates were lost due to prolonged flooding. The peanut crop was harvested on 7 October 2004. Data collected included J2 per 100 cm³, endospore per J2, percentage infected females, root and pod gall indices.

A winter cover crop, common hairy vetch (*Vicia villosa* Roth), was planted on 21 October 2004 and harvested 8 April 2005. Production practices common for growing vetch for the area were applied (Undersander et al., 1990). Data collected at the harvest of the vetch crop included J2/100 cm³, endospores/J2, percentage infected females, root galling and egg mass indices.

In spring 2005, following the harvest of the winter vetch crop, the fumigant treatments were again applied in the same plots as in 2004. Peanut cv. Georgia Green was seeded on 10 May 2005. At the end of the peanut crop, data were collected as previously described above. In addition, subjective plant growth rating and peanut yield data were collected per plot. The peanut growth ratings were scored on a scale of 1 to 10 (1 = poor growth and 10 = good growth) (Cetintas and Dickson, 2004).

**Statistical Analysis**

Data were subjected to analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC) and mean treatment differences were separated and compared using Waller-Duncan \( k \) ratio \((k = 100)\) \( t \)-test. Microsoft Excel (Microsoft, Redmond, WA) was used for regression analysis. Data for number of J2 per 100 cm³ of soil and endospore
per J2 were transformed using \( \log_{10}(x+1) \) before statistical analysis. Untransformed numbers are presented in the text and tables.

Results

Transfer and Establishment of *P. penetrans*

In spring 2003, a soil bioassay was used to show the levels of *P. penetrans* endospores per J2 after introduction was 1.3 ± 0.7 endospore/J2. In the autumn, following the first peanut harvest, endospores of *P. penetrans* were found attached to J2 with a mean number of 1.1 ± 1.0. Following a winter wheat crop, spring 2004, the mean number of endospores per J2 had increased to 5.0 ± 2.2. These means were based on total plots \((n = 54)\).

The Effects of Soil Fumigants on *P. penetrans* Development

In autumn 2004, after peanut harvest, there were differences between nontreated (*P. penetrans* and *M. arenaria* infested plots) and the fumigant-treated plots (1,3-D and chloropicrin treated plots), with respect to J2 per 100 cm\(^3\) of soil, endospores per J2, percentage infected females, and root gall indices \((P \leq 0.05)\) (Table 3.1). The highest number of J2 was found in the chloropicrin-treated plots followed by the nontreated and 1,3-D treated plots \((P \leq 0.05)\) (Table 3.1). The nontreated plots had higher number of endospore encumbered J2 than the chloropicrin and 1,3-D treated plots \((P \leq 0.05)\). The highest percentage of infected females was observed in nontreated plots, followed by 1,3-D and chloropicrin treated plots \((P \leq 0.05)\) (Table 3.1). The root-gall index was highest in nontreated plots followed by chloropicrin and 1,3-D treated plots \((P \leq 0.05)\) (Table 3.1). The pod galling indices were highest in nontreated plots compared with the 1,3-D treated plots \((P \leq 0.05)\) (Table 3.1). A linear regression model \( Y = 0.958x + 29.001, r^2 = 0.0132, P = 0.5046 \) of the relationship between endospore numbers per J2 in spring...
before planting peanut and percentage infected females at harvest in autumn, 2004 was not significant ($P \leq 0.05$) (Fig. 3.4).

In spring 2005, after vetch harvest, the highest numbers of J2 were in the chloropicrin treated plots, followed by 1,3-D and the nontreated plots ($P \leq 0.05$) (Table 3-2). Mean endospores per J2 was highest in nontreated plots followed by 1,3-D and chloropicrin treated plots ($P \leq 0.05$) (Table 3-2). The percentage infected females was highest in the nontreated plots, which differed from chloropicrin and the 1,3-D plots ($P \leq 0.05$). There were no differences between chloropicrin and the 1,3-D treated plots. For both root galling and egg mass indices, the highest numbers were recorded in the chloropicrin treated plots, followed by 1,3-D treated plots and lowest in the nontreated plots. The latter two were not different ($P \leq 0.05$) (Table 3-2).

In autumn 2005, after peanut harvest, the highest number of J2 per 100 cm$^3$ was again recorded in the chloropicrin treated plots ($P \leq 0.05$) (Table 3.1). There were no differences between the number of J2 extracted from the nontreated and 1,3-D plots ($P \leq 0.05$) (Table 3.1). The endospore per J2 were high in the nontreated plots followed by 1,3-D treated plots and chloropicrin treated plots. The latter two were not different ($P \leq 0.05$).

The percentage of J2 with endospores attached (native bioassay) was highest in nontreated control followed by 1,3-D treated plots and lowest in chloropicrin treated plots, all three were different ($P \leq 0.05$) (Fig. 3-1). The results were in agreement with that of the percentage infected females, where the highest percentage infected females were in the nontreated plots followed by 1,3-D treated plots and then chloropicrin treated plots. All the treatments were different ($P \leq 0.05$) (Fig. 3-1). The nontreated control had
the lowest numerical root gall index. The highest root gall index was in the chloropicrin treated plots. The same trend was observed for the pod gall index where there were no differences between nontreated plots and 1,3-D treated plots, but the two were different from the chloropicrin treated plots \( (P \leq 0.05) \), which had the highest number. A regression model, \( Y = 12.683 \ln(x) + 26.773 \), \( r^2 = 0.2499 \), \( P = 0.0019 \), to explain the relationship between endospore per J2 in spring before a peanut crop to percentage infected females in autumn at harvest of the peanut crop was significant \( (P \leq 0.05) \) (Fig. 3.4).

The plant growth indices of the peanut crop in the field were low in chloropicrin treated plots but high in both the nontreated plots and the 1,3-D treated plots. The latter two were not different \( (P \leq 0.05) \) (Figs. 3.2; 3.5; 3.6). The nontreated plots and 1,3-D treated plots had the highest peanut yield per plot \( (P \leq 0.05) \). Chloropicrin treated plots had lower yields than nontreated or 1,3-D treated plots \( (P \leq 0.05) \) (Fig. 3.3).

**Discussion**

Transfer and Establishment of *Pasteuria penetrans* and *Meloidogyne arenaria* race 1

The results demonstrated that *P. penetrans*-infested dried peanut pods, pegs and roots can be transferred from one site to another and the bacterium amplified to suppressive levels against the peanut root-knot nematode. Care should be taken to ensure that the peanut pegs and pods are left to dry for at least 2 weeks so that live nematodes and other live disease propagules are not transferred alongside the *Pasteuria* infested propagules. Endospores of *P. penetrans* were reportedly killed by autoclaving but only slightly affected by microwaving (Weibelzahl-Fulton, 1998) and it was expected that *P. penetrans* endospores would withstand the relatively high temperatures such as those that occur in Florida during the summer months, whereas nematodes in the exposed roots
would not. Endospores of *P. penetrans* resist various chemicals and stressful environmental conditions (Mani, 1988; Williams et al., 1989).

After the transfer of *P. penetrans* and subsequent introduction of *M. arenaria*, a peanut crop was planted to amplify both the *P. penetrans* and *M. arenaria*. Surprisingly there were no significant differences between endospores per J2 in the soil before and immediately after peanut harvest during the first year. During the first peanut season, the endospore density in the soil was lowest at peanut harvest. However, after a wheat crop in the spring of 2004, the endospore density in the soil increased exponentially. A possible explanation is that by peanut harvest most of the endospores were still held within the females that were still residing within roots. It is only after the roots decompose that the females and subsequently the endospores would be released in to soil. In this study, this occurred in the spring of the following year. Hence, to correctly estimate endospore density in soil following a peanut crop, the best time to sample may be in early spring rather than immediately after a peanut harvest. This is a departure from tradition where nematode and endospore density in the soil are usually determined immediately after the harvest of the crop.

The wheat winter cover crop planted in autumn of 2003 had very few discernable galls. The wheat cultivar was tested as a host for *M. arenaria* race 1 in the greenhouse and proved to be a poor host. Even if the winter crop was a susceptible host to *M. arenaria* race 1, existing high number of endospores density in the soil would likely have attached to the juveniles and hindered their mobility and subsequent infection of the wheat roots. Reductions in plant infectivity have been observed when nematodes are encumbered with 15 to 20 endospores (Brown and Smart, 1985). In this study as few as
three endospores per juvenile was shown to interfere with J2 penetrating tomato roots (chapter 1).

Effects of Soil Fumigants on *Pasteuria penetrans*

Applying 1,3-D and chloropicrin allowed the evaluation of the effects of fumigants on *P. penetrans*. The effective role of 1,3-D as a soil fumigant against *M. arenaria* was evident, resulting in the lowest J2 per 100 cm$^3$ of soil being detected in 2004 and 2005 after peanut. The fact that 1,3-D did not have an adverse effect on the percentage infected females supports the hypothesis that 1,3-D can be used alongside *P. penetrans* to manage *M. arenaria*. The differences in *P. penetrans* population densities in 1,3-D plots and the nontreated control could be attributed to the fact that when 1,3-D was applied, a high percentage of J2 in the 0 to 20 cm depth were killed, thus limiting development of *P. penetrans*. Infection that occurred later could have resulted from re-infestation of J2 from below the fumigated soil or from nematode eggs that had not hatched in time to be affected by the fumigant. Prot (1978) reported that J2 of *Meloidogyne* spp. have the ability to migrate vertically up to 50 cm. This movement can be attributed to thermal gradients (Diez and Dusenbery, 1989). Computer modeling studies suggest that, the thermal dynamics in the soil environment would cause the nematodes to move toward the soil surface (Dusenbery, 1988). In this study such a movement renders the J2 available in the root rhizosphere where the majority of endospores are concentrated. The juveniles that become encumbered with *P. penetrans* endospores are needed in order for further amplification of *P. penetrans*.

Chloropicrin was detrimental to *P. penetrans* as the lowest percentage of infected females, endospore per J2, and highest nematode density in soil were recorded with chloropicrin treated plots. Gall indices were greater in chloropicrin treated plots.
compared to the nontreated plots and 1,3-D treated plots in the second season as opposed to the first season. These data support the hypothesis that chloropicrin is detrimental to *P. penetrans* not necessarily at the level of attachment, but at the level of development. Freitas (1997) reported that different formulations of 1,3-D and chloropicrin had moderate effects on *P. penetrans*. Our study confirms that chloropicrin is responsible for the bactericidal effect.

The highest percentage of infected females in nontreated plots differed from 1,3-D treated plots and chloropicrin treated plots. In terms of yields there was no significant differences between 1,3-D treated plots and nontreated control plots. *P. penetrans* development in nontreated plots risks achieving soil suppressiveness, which decreases further amplification. The results of these studies demonstrate that the degree of control of *M. arenaria* in the nontreated control (infested with both *P. penetrans* and *M. arenaria*) was similar to that of the 1,3-D treated plots. This is an important indication that 1,3-D could be used alongside *P. penetrans*, especially in fields highly infested with nematodes where 1,3-D controls the nematode population during the first year and later *P. penetrans* is allowed to establish since it is known to be density dependent. Cultivation of a susceptible host crop for more than one season is needed for *P. penetrans* to build up densities to suppressive levels (Melki et al., 1998).

Attempts to predict the level of suppressiveness based on the endospore per J2 in the soil using a regression model was not successful during the second year following peanut, but the model was significant after the third year following peanut. The use of the artificial bioassay, which involves drying to kill all the nematodes and then re-introducing J2 gives better detection of *P. penetrans* than extracting endospore-
encumbered J2 from soil (Cetintas and Dickson, 2004). Air drying of soil could inhibit other microbial activity that could be antagonistic to endospore attachment, or it may be a matter of maturation of endospores. It is known that shedding the exosporium coat increases the ability of endospores to attach to the nematode cuticle (Davies et al., 1988; Stirling, 1985). Detecting endospore densities in the soil with precision before planting a crop or early in the growing season may help growers to know whether or not to apply other management practices especially when the crop being planted is susceptible to the nematode in question.

In summary, *P. penetrans* can be transferred from one site to another and become established once a nematode host and a crop susceptible to the nematode host are introduced. *P. penetrans* is not affected adversely by application of the soil fumigant 1,3-D however, chloropicrin interfered with *P. penetrans* development in root-knot nematode females.
Table 3-1. Effect of chloropicrin and 1,3-D on *Pasteuria penetrans* and *Meloidogyne arenaria* race 1 following two seasons of a peanut crop, 2004 and 2005, and a winter cover crop of vetch 2004-05. All plots were infested with *P. penetrans* and *M. arenaria* including the nontreated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>J2/100 cm³</th>
<th>Endo./J2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% IF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Root GI&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Pod GI&lt;sup&gt;c&lt;/sup&gt;</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn 2004 (at peanut harvest)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>84.7 b</td>
<td>7.4 a</td>
<td>49.0 a</td>
<td>2.6 a</td>
<td>2.6 a</td>
<td></td>
</tr>
<tr>
<td>Chloropicrin</td>
<td>144.8 a</td>
<td>2.4 b</td>
<td>18.8 c</td>
<td>1.8 b</td>
<td>1.9 ab</td>
<td></td>
</tr>
<tr>
<td>1,3-D</td>
<td>30.2 c</td>
<td>2.4 b</td>
<td>32.6 b</td>
<td>1.1 c</td>
<td>1.6 b</td>
<td></td>
</tr>
<tr>
<td>Spring 2005 (at vetch harvest)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>16.8 c</td>
<td>2.0 a</td>
<td>26.7 a</td>
<td>1.5 c</td>
<td>–</td>
<td>1.2 c</td>
</tr>
<tr>
<td>Chloropicrin</td>
<td>79.7 a</td>
<td>0.7 b</td>
<td>4.1 b</td>
<td>3.7 a</td>
<td>–</td>
<td>4.1 a</td>
</tr>
<tr>
<td>1,3-D</td>
<td>39.0 b</td>
<td>1.2 b</td>
<td>7.5 b</td>
<td>2.6 b</td>
<td>–</td>
<td>3.0 b</td>
</tr>
<tr>
<td>Autumn 2005 (at peanut harvest)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>63.6 b</td>
<td>3.6 a</td>
<td>54.9 a</td>
<td>1.1 b</td>
<td>1.1 b</td>
<td></td>
</tr>
<tr>
<td>Chloropicrin</td>
<td>152.1 a</td>
<td>0.5 b</td>
<td>22.1 c</td>
<td>4.2 a</td>
<td>4.1 a</td>
<td></td>
</tr>
<tr>
<td>1,3-D</td>
<td>51.6 b</td>
<td>1.4 b</td>
<td>47.4 b</td>
<td>1.4 b</td>
<td>1.3 b</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of 12 replicates; means within a column followed by the same letter(s) are not significantly different according to Waller-Duncan k ratio (k = 100) t-test (P ≤ 0.05). Data for J2 per 100 cm³ of soil and endospore per J2 were transformed using log<sub>10</sub> (x+1) before statistical analysis. Untransformed numbers are presented. No fumigants were applied in nontreated control plots.

<sup>a</sup> The first 20 second-stage juveniles (J2) were observed for the presence of endospores attached to the nematode cuticle.

<sup>b</sup> Percentage infected females was based on the first 30 females observed for the presence or absence of endospores inside their body.

<sup>c</sup> Root gall, pod, and egg mass (EM) indices were rated using a 0-5 scale where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = more than 100 galls (Taylor and Sasser, 1978).
Fig. 3-1. Effect of chloropicrin (pic) and 1,3-D on percentage of *Pasteuria penetrans* infected *Meloidogyne arenaria* race 1 females (%IF) and second-stage juveniles (J2) with endospores attached (%IJ) of *Meloidogyne arenaria* race 1 at harvest of the second peanut crop, September 2005. All plots were infested with *P. penetrans* and *M. arenaria* including the nontreated control.
Fig. 3-2. Effect of chloropicrin (pic) and 1,3-D on plant growth of peanut grown in a site infested with both *Pasteuria penetrans* and *Meloidogyne arenaria* race 1, one week before the harvest of the second peanut crop, September 2005. Plant growth index rated on a scale of 1-10 (1 = poor growth, 10 = good growth). All plots were infested with *P. penetrans* and *M. arenaria* including the nontreated control.
Fig. 3-3. Effect of chloropicrin (pic) and 1,3-D applied after the transfer of *Pasteuria penetrans* on peanut yield (kg) per plot at harvest of the second peanut crop, September 2005. All plots were infested with *P. penetrans* and *M. arenaria* including the nontreated control.
Fig. 3-4. Effect of *Pasteuria penetrans* endospore density in soil in spring before a peanut crop on the percentage infected females in fall after peanut harvest. A) Correlation of endospores per J2 in soil before planting spring 2004 to percentage *P. penetrans* infected females (IF) at harvest in autumn 2004 was not significant (*P* ≤ 0.05). B) Regression of endospore per J2 before planting a peanut crop in spring 2005 to percentage infected females after the harvest of the peanut crop in autumn 2005 (*P* ≤ 0.05).
Fig. 3-5. Effect of soil fumigants and *Pasteuria penetrans* on peanut growth. A) Nontreated control peanut plot and B) 1,3-D treated plot 115 days after planting. The nontreated plots were similar with the 1,3-D treated plots in terms of plant growth ratings (*P* ≤ 0.05). All plots were infested with *P. penetrans* and *M. arenaria* including the nontreated.
Fig. 3.6. Effect of soil fumigants and *Pasteuria penetrans* on peanut growth. A) Nontreated control and B) chloropicrin treated plot 115 days after planting. There were differences between the growth ratings of both 1,3-D and nontreated control and those of chloropicrin treated plots ($P \leq 0.05$). All plots were infested with *P. penetrans* and *M. arenaria* including the nontreated.
CHAPTER 4
THE INFLUENCE OF HOST CROPS AND DIFFERENT DENSITIES OF Meloidogyne arenaria RACE 1 ON AMPLIFICATION OF Pasteuria penetrans

Introduction

Pasteuria penetrans (Thorne) Sayre & Starr is an obligate, endospore-forming gram positive bacterium considered to be a promising biological control agent for root-knot nematodes, Meloidogyne spp. (Dickson et al., 1994; Mankau, 1980; Sayre and Starr, 1988; Stirling, 1985). It has been shown to be effective in controlling root-knot nematodes in field and microplot studies (Bekal et al., 2000; Brown and Smart, 1985; Chen and Dickson, 1998; Chen et al., 1996b; Dickson et al., 1994; Freitas et al., 2000; Minton and Sayre, 1989; Oostendorp et al., 1990; 1991; Stirling, 1984; Stirling, 1991; Weibelzahl-Fulton et al., 1996). P. penetrans has been consistently associated with nematode suppressive soils (Bird and Brisbane, 1988; Dickson et al., 1991, 1994; Mankau, 1980; Minton and Sayre, 1989) and the suppressiveness has been successfully tested in greenhouse studies (Dube and Smart, 1987; Stirling, 1984).

Pasteuria penetrans has not been grown successfully on artificial media because it is an obligate parasite of root-knot nematode. Currently, it must be cultivated on nematodes in the greenhouse (Stirling and Wachtel, 1980) or in excised root systems (Verdejo and Jaffee, 1988). The use of P. penetrans on a commercial scale will require an in vitro method of cultivation. Various media have been tested for artificial cultivation of Pasteuria spp. but without success (Bishop and Ellar, 1991; Reise et al., 1988; Williams et al., 1989).
The population dynamics of *P. penetrans* has not been fully elucidated. It has been demonstrated that when *P. penetrans* is introduced into a soil containing damaging densities of *M. arenaria*, the bacterium will amplify to densities that are suppressive to the nematode within 3 years (Oostendorp et al., 1990, 1991), or sooner if high densities of endospores (100,000/g of soil) are added (Chen et al., 1996a). Microplot studies demonstrated that cropping sequence of peanut and winter cover crops increased infection of *M. arenaria* by *P. penetrans* and improved peanut growth over time (Oostendorp et al., 1990). Planting a root-knot nematode susceptible crop during summer months increased *P. penetrans* more than susceptible winter cover crops.

There are likely many factors that affect endospores in soil suppressive sites. Endospores have been observed being devoured by protozoa in laboratory dishes (Chen and Dickson, 1998) but nothing is known about them serving as a food base for protozoa or other organism in soil. There is a known downward dispersal of endospores with percolating water that could result in their depletion from the upper soil horizon if they are not being continuously amplified in this zone (Cetintas and Dickson, 2005).

There are at least two phases to the mode of action of the bacterium in causing soil suppressiveness: endospore-encumbered J2 mobility is reduced, female nematode fecundity is significantly reduced. For example, in soils heavily infested with *P. penetrans* endospores, a large number of migratory J2 become encumbered with endospores thereby becoming less mobile, thus fewer J2 enter roots (Davies et al., 1991;; Mankau and Prasad, 1972). Endospore-encumbered J2 that enter roots establish feeding sites, develop to mature females, but these females do not produce eggs (chapter 1; Gowen et al., 1989; Sayre, 1980). As a result of these actions, the natural infestations of
P. penetrans become sufficiently high to suppress populations of Meloidogyne spp. (Bird and Brisbane, 1988; Dickson et al., 1994; Minton and Sayre, 1989; Weibelzahl-Fulton et al., 1996; Timper et al., 2001).

Since P. penetrans is density dependent, its development depends on infection of a host nematode and that nematode’s development within a plant. However, with reduced J2 mobility and reduced fecundity continued amplification of the bacterium is difficulty (Cetintas and Dickson, 2005). This may require that P. penetrans host nematode densities be maintained at low densities in order to maintain soil suppressiveness (Cetintas and Dickson, 2005). The influence of different summer crops and different densities of root-knot nematode juveniles on the abundance and amplification of P. penetrans has not been documented. The objective of the experiment was to compare the effects of different summer crops and different nematode densities on the abundance and amplification of P. penetrans.

Materials and Methods

Transfer and Establishment of Pasteuria penetrans and Meloidogyne arenaria in Microlplots

The study was carried out in microplots located at the University of Florida, Gainesville, FL. The microplots measured 270 cm long x 60 cm wide x 60 cm deep and were prepared for inoculation in autumn 2002. Inoculum of P. penetrans isolate P20 was collected from a known M. arenaria soil suppressive site located in Levy Co., FL (Dickson et al., 1994). In October 2002 galled peanut roots, pegs and pods were dug, and left on soil surface for 2 weeks to dry. Approximately 5 kg of dried roots, pegs, and pods were spread over the soil surface of 12 microplots and incorporated into the top 30 cm of soil. On 22 March 2003, inoculum of M. arenaria was added to each microplot. The
culture of *M. arenaria* originated from peanut grown at former University of Florida Green Acres Agronomy Farm, Alachua Co., FL and was maintained in a greenhouse on tomato (*Lycopersicum esculentum* M. cv. Rutgers). *M. arenaria* was introduced from infested soil and galled tomato roots. The inoculum, which was equally distributed in the microplots and covered, was grown on tomato cv. Rutgers) in 15-cm-diam. pots.

**Soil Bioassay for Detection of *Pasteuria penetrans* in the Soil**

A soil bioassay was carried out on 22 March 2003 to quantify the *P. penetrans* endospore density per microplot (Oostendorp et al., 1990). Five soil cores per microplot were taken with a cone-shaped sampling tube (2.5-cm-diam., 20-cm-deep). Forty grams from each sample was placed in a small dish on lab bench to air dry for 2 weeks. This was then placed into a 50-ml sterile polyethylene centrifuge tube, and the moisture content adjusted to 100% field capacity (saturated) to increase the rate of endospore attachment (Brown and Smart, 1984). Then, 500 (1 to 3-day-old) J2 of *M. arenaria* were added and the tubes left uncovered at room temperature. Three days later, the J2 were extracted by centrifugal-flotation method (Jenkins, 1964), and the first 20 J2 per sample were observed for number of endospores attached at 200× magnification. Also, the number of J2 was determined by extracting them from 100 cm³ of the same soil samples as described above.

Peanut (*Arachis hypogaea* L. cv. Georgia Green) was planted on 17 April 2003 in all microplots to serve as a host plant for *M. arenaria* and subsequently the amplification of *P. penetrans* endospores. Each microplot was planted with 36 peanuts seeds in three rows spaced 32-cm apart. Agronomic practices common for growing peanut in the region were applied (Whitty, 2002). On 3 September 2003 peanut roots were inverted, left on soil surface for 2 weeks to dry before they were incorporated into the top 30 cm of
soil. Five soil cores were taken from each microplot as previously described above and evaluated for numbers of J2 and endospores per J2.

On 7 November 2003 a wheat cover crop (*Triticum aestivum* L. cv. AGS 2000) was seeded in all microplots in five rows spaced 18-cm apart. A total of 60 plants were planted per microplot. The wheat roots were dug on 21 April 2004 and allowed to air dry for 2 weeks before they were incorporated into the top 30 cm of soil. Soil samples for determining number of J2 per 100 cm³ of soil and endospores per J2 in each of the microplot were collected as described above. These numbers formed the baseline population densities of *P. penetrans* and *M. arenaria* for the next experiment on the effect of nematode densities and summer crops on *P. penetrans* amplification.

**Effect of Nematode Densities and Different Summer Crops on *Pasteuria penetrans*—Spring-Autumn 2004**

Each microplot was divided with metal barriers into three subplots measuring 90 cm × 60 cm × 60 cm for a total of 36 plots. The experiment was a 2 × 3 factorial arranged in a randomized complete block design replicated six times. Three nematode densities (J2), low, medium, and high (4, 20, and 40 J2/100 cm³ of soil, respectively) of *M. arenaria* J2 were introduced in the microplots on 21 May 2004. The J2 were suspended in tap water and inoculated by pipetting to ensure uniform distribution across the microplots. The numbers of J2 added were based on the top 30 cm of soil for each microplot. The two summer crops were peanut cv. Georgia Green (135-day crop) and squash (*Cucurbita pepo*) cv. Yellow Crookneck (55-day crop). Each microplot was either planted with 12 peanut plants or four squash plants. Two crop cycles of squash (short season) were compared with one crop cycle of peanut (long season). The first cycle of squash roots were placed back into the soil and the second cycle squash planted
2 weeks later. Agronomic practices for squash and peanut followed recommended practices (Olson et al., 2005; Whitty, 2002).

On 16 September 2004, the plant roots of both crops were dug. Data collected included J2 per 100 cm³ of soil, endospores per J2, root galling and egg mass indices, and percentages of infected females as described in chapter 3. Gall and egg mass indices were rated using a 0 to 5 scale where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses, 2 = 3-10 galls or egg masses, 3 = 11-30 galls or egg masses, 4 = 31-100 galls or egg masses, and 5 = >100 galls or egg masses per root system (Taylor and Sasser, 1978). Egg masses were stained using a red-food coloring technique (Thies et al., 2002).

Female root-knot nematodes were extracted from roots by subjecting them to digestion by placing them in a 1,800-ml beaker containing 10% Rapidase Pomaliq 2F (Gist-Brocades Pomaliq product number 7003-A/DSM, Food Specialties USA, Menominee, WI), 50 mM NaAcetate (pH 5.0), and 0.1% CaCl₂ at ca. 50:50 (v/v), and placed at room temperature (Charnecki, 1997) The roots were digested for 3 days to free endospore-filled females from root galls. The females were collected by decanting onto a 600-µm-pore sieve nested on a 150-µm-pore sieve series and subjected to a high pressure spray of water in order to dislodge females. Thirty females were hand-picked with forceps and examined for the presence or absence of mature endospores at 400× magnification.

On 29 October 2004, a winter cover crop of vetch (Vicia villosa Roth) was planted based on the broadcast seeding rate. The vetch was dug and roots were incorporated into the soil 5 April 2005. Root and soil samples were collected as described above.
Effect of Nematode Densities and Different Summer Crops on *Pasteuria penetrans*—Spring-Autumn 2005

The experiment was repeated in 2005. During the second squash cycle, due to vulnerability of the maturing peanut pods to raccoons, the experiment was terminated July 2005. The above-ground portions of all plants were cut, and the roots, pegs and pods were turned under in the top 30 cm of soil. Okra (*Abelmoschus esculentus* L. Moench cv. Hybrid Okra Sok 6101) was seeded in all plots 15 August to obtain a final stand of six okra plants per microplot. Cultural practices, fertilization, and pest management for okra was as recommended for Florida (Aerts and Mossler, 2005; Kemble, 1995). Okra fruit was harvested over a 1-month period and roots were dug 1 November 2005. Data collected included J2 per 100 cm³ of soil, endospores per J2, gall and egg mass indices, percentage infected females, shoot and root fresh and dry weights.

**Data Analysis.**

All data were subjected to analysis of variance (ANOVA) by using the GLM procedure in SAS/STAT version 9.1 (SAS Institute, Cary, NC). If the treatment effects were significant (*P* ≤ 0.05) means were separated by Waller-Duncan *k* ratio (*k*=100) *t*-test. Data on J2 per 100 cm³ and endospore per J2 were transformed using log₁₀(*x*+1) before statistical analysis. Untransformed values are presented in the text, tables and figures.

**Results**

**Transfer and Establishment of *Pasteuria penetrans* and *Meloidogyne arenaria* in Microplots**

The average number of endospores attached per J2 in spring 2003 after the harvest of the wheat crop was 4.5 ± 1.7. Following peanut in autumn the number had increased to 11.4 ± 1.8 endospores/J2 and increased again following wheat harvest in spring 2004
to 43.4 ± 3.8. Two weeks following *M. arenaria* inoculation into microplots an average of 5.3 ± 0.3 J2/100 cm³ of soil was extracted. Following peanut harvest in autumn the number extracted was 142.6 ± 6.2 J2/100 cm³ of soil. This number decreased to 18.8 ± 2.0 J2/100 cm³ of soil following wheat harvest in spring 2004.

**Effect of Nematode Densities and Different Summer Crops on *Pasteuria penetrans*—Spring-Autumn 2004**

There was no significant interaction between crop type and nematode densities among the parameters evaluated. Endospore per J2 were not affected by the number of J2 that was added to each microplot, however, there were greater numbers of J2 per 100 cm³ of soil, increased root galling, but fewer endospore-filled females where high densities of J2 were added (*P* ≤ 0.05) (Table 4-1). The number of J2, galls, and endospore-filled females were not different between the low and medium nematode densities (*P* ≤ 0.05). There were greater endospores per J2, number of J2 per 100 cm³, and greater root galling in one cycle of peanut as compared to two cycles of squash (*P* ≤ 0.05) (Table 4-1). The percentages of endospore-filled females were not affected by crop type and nematode × crop interaction. The highest percentages of endospore-filled females were in the low and medium nematode densities. There were no differences in the percentage of endospore-filled females between low and medium densities (*P* ≤ 0.05) (Table 4.1). Root galling was greater in peanut than squash (*P* ≤ 0.05) (Table 4.1). Galling was increased under high nematode densities.

There was no significant interaction between crop type and nematode densities regarding number of J2 per 100 cm³ of soil and root galling and egg mass indices, however there was a significant interaction relative to crop type and nematode densities on number of endospores recovered (*P* ≤ 0.05) (Table 4-2, 4-3) in spring 2005 after the
harvest of the vetch winter crop. The latter was analyzed separately. The number of J2 per 100 cm$^3$ of soil were not different among treatments, but number of galls and egg masses were greater where the highest nematode level was applied and where squash had been planted in the previous crop season ($P \leq 0.05$) (Table 4-2). Data on percentage of endospore-filled females were not included because no mature female nematodes were recovered. Endospore numbers increased in the former peanut and squash plots that had high densities of J2 added as compared to where medium and low densities were added ($P \leq 0.05$) (Table 4-3).

Following okra harvest November 2005, there was a significant interaction relative to crop type and nematode densities on number of endospores and J2 per 100 cm$^3$ of soil ($P \leq 0.05$) (Table 4-4). There was large increase in number of endospores per J2 in the high and medium J2 infested former peanut plots compared with plots containing low J2 densities ($P \leq 0.05$) (Table 4-4, Fig. 4.1). Correspondingly there was no increase in the numbers of J2 per 100 cm$^3$ among the three nematode densities. No differences were detected among the three nematode densities in former squash plots, however there was a spike in the number of J2 per 100 cm$^3$ of soil recovered in the high nematode infested plots ($P \leq 0.05$) (Table 4-4).

Former peanut plots had the lowest mean gall index compared with former squash plots ($P \leq 0.05$) (Table 4.5). Root galling was greater in the medium and high nematode densities compared with the low nematode densities ($P \leq 0.05$). So few galls were detected in former peanut plots that it was impossible to determine the percentage endospore-filled females, whereas former squash plots had a high level of galling with high incidence of endospore-filled females ($P \leq 0.05$) (Table 4-5, Fig. 4-2).
There was no significant interaction between crop type and nematode densities among the parameters evaluated following okra harvest \((P \leq 0.05)\) (Table 4-6). Nematode densities had no effect on parameters tested, but where okra was grown following peanut there was greater mean shoot fresh and dry weight, dry root and shoot weights, and fruit was nearly double that from squash plots \((P \leq 0.05)\) (Table 4-6).

**Discussion**

*Pasteuria penetrans* and *M. arenaria* were successfully established and amplified in microplots. The peanut crop planted in spring 2003 served to increase both *M. arenaria* and *P. penetrans*. The high number of J2 per 100 cm\(^3\) of soil at the end of the peanut crop in 2003 is consistent with previous reports that root-knot nematodes multiply rapidly on this highly susceptible host. The continuous growth of a root-knot susceptible host that has a relative long crop season may be the key to developing soil suppressiveness (Bird and Brisbane, 1988; Dickson et al., 1994; Madula et al., 1994). In nature, mutual selection is reported to produce a dynamic balance between densities of *P. penetrans* and root-knot nematodes whereby the bacterium rarely becomes suppressive (Trudgill et al., 2000). However, some authors have reported a high degree of natural soil suppressiveness to root-knot nematodes due to *P. penetrans* build up (Bird and Brisbane, 1988; Dickson et al., 1991; 1994; Minton and Sayre, 1989).

During the first two seasons the highest densities of *M. arenaria* in the soil were always at peanut harvest however, during the third crop season the number of juveniles extracted from peanut soil decreased. There was a corresponding increase in the number of endospores per J2. However, they remained relatively low until the third crop season. The addition of relatively high densities of healthy J2 increased the number of endospores per J2 in all plots regardless of the nematode host crop. It is likely that at
crop harvest most endospores were still captured within female cadavers inside the roots. It is only after the roots decomposed and females cadavers were released in soil did endospore densities increase. This is an important aspect that needs to be factored in the population dynamics of *P. penetrans* and *M. arenaria*.

After 2004, the highest percentage of endospore-filled females was observed in low nematode density plots under a peanut summer crop. However, similar observations were not noted in 2005, where few galls or females could be found in peanut plots because *P. penetrans* densities had reached suppressive densities. This confirms that ca. 3 years are required to develop *P. penetrans* to suppressive densities. Suppressiveness builds slowly because of the density dependent relationship between *P. penetrans* and root-knot nematode. This is an indication that soil suppressiveness due to *P. penetrans* takes more than 1 or 2 years to develop.

The highest percentage infected females in squash plots was detected in the high nematode density plots. In autumn 2005, at okra harvest, higher number of endospores per J2 corresponded to greater nematode densities, which were greater in peanut than squash plots. This study confirmed the hypothesis that *P. penetrans* will increase quickly where there is a high initial density of root-knot nematodes J2, and where a long season susceptible crop is grown. Similar results have been made by Darban et al. (2005) and Madula et al. (1994). Brinjal followed by mungbean, mash and cowpea were suitable crops suggested for increasing production of *P. penetrans* (Javed et al., 2002). It was postulated that the increase in endospore density with brinjal was due to greater available infection sites for root-knot nematodes. There was a positive relationship between quantity of roots and the amount of endospores produced. With a 135 day peanut crop
there are at least 5 to 6 generations of *M. arenaria* (Davila, pers. comm.). Also, pegging and pod set provides for additional root-knot nematode infection sites, which aids *P. penetrans* development (Dickson and De Waele, 2005).

The long season peanut crop compared to the much shorter season for squash (ca. 55-days) provides ample time for the *P. penetrans* to develop and sporulate. Squash may not have provided this opportunity because by the time the crop is destroyed *P. penetrans* in some infected females may not have reached maturity. Peanut may be an ideal crop for amplifying *P. penetrans* to suppressive densities because it is grown in a hot climate and is a long season crop. Both conditions favor development of *P. penetrans* (Hatz and Dickson, 1992; Serracin et al., 1997). Where peanut has been grown in sites infested with both *M. arenaria* and *P. penetrans* the bacterium causes almost complete protection of roots, pegs and pods from galling. Once J2 densities in the soil are reduced, *P. penetrans* density may also diminish due to leaching and other factors yet to be documented (Cetintas and Dickson, 2005). It is unknown how long soil suppressiveness can be sustained, but it is known that suppressiveness can be lost when one species of root-knot nematode is replaced by another species. Suppressiveness of *M. arenaria* by a population of *P. penetrans* specific to the species is apparently being lost because of a build up of *M. javanica*. The latter is not affected by the *P. penetrans* population (Cetintas and Dickson, 2005).

In order to maintain and amplify an isolate of *P. penetrans* for experimental purposes, a certain number of the nematode host must be added periodically. Otherwise the bacterium will drive the nematode population to undetectable levels. A certain
number of juveniles must always escape infection in the soil so as to produce more eggs and subsequent juveniles for further amplification of *P. penetrans*.

*Pasteuria penetrans* has great potential for integration with other cultural or nematode management practices (Brown and Nordmeyer, 1985; Oostendorp et al., 1991; Walker and Wachtel, 1989) and there is need to select nematode management practices that are complimentary to the suppressiveness with no known adverse effect on *P. penetrans*. This study demonstrates that the most effective plant host for amplification of *P. penetrans* isolate P20 from *M. arenaria* is a peanut crop under high nematode densities. It must be pointed out that even when high nematode densities (40 J2 100 cm³ of soil) were added to the plots, the soil became suppressive to *M. arenaria*. Additional long-term experiments are needed to determine the effect of nematode densities and summer cover crops on the amplification of existing *P. penetrans* densities beyond the duration of this experiment.
Table 4-1. Effect of one crop cycle of peanut vs. two crop cycles of squash and *Meloidogyne arenaria* race 1 densities on mean number of endospores per second-stage juvenile (J2), J2 per 100 cm$^3$ of soil, gall index, and percentage endospore-filled females during a spring-autumn 2004 cropping period.

<table>
<thead>
<tr>
<th>Nematode densities</th>
<th>Endo./J2$^a$</th>
<th>J2$^b$</th>
<th>GI$^c$</th>
<th>IF$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (4 J2)</td>
<td>6.3 a</td>
<td>39.0 b</td>
<td>0.9 b</td>
<td>47.0 a</td>
</tr>
<tr>
<td>Med. (20 J2)</td>
<td>7.8 a</td>
<td>59.5 b</td>
<td>0.5 b</td>
<td>30.8 a</td>
</tr>
<tr>
<td>High (40 J2)</td>
<td>7.3 a</td>
<td>149.4 a</td>
<td>1.1 a</td>
<td>26.5 b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crops</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>8.4 a</td>
<td>143.1 a</td>
<td>1.1 a</td>
<td>39.1 a</td>
</tr>
<tr>
<td>Squash</td>
<td>5.9 b</td>
<td>22.2 b</td>
<td>0.5 b</td>
<td>30.4 a</td>
</tr>
</tbody>
</table>

Means are an average of six replications. Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Waller-Duncan $k$ ratio ($k=100$) $t$-test.

$^a$ Number of endospores attached per J2 was counted based on the first 20 J2 observed per sample.

$^b$ J2 per 100 cm$^3$ of soil extracted using centrifugal-flotation technique (Jenkins, 1964).

$^c$ Gall index rating based on a 0-5 scale where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = more than 100 galls (Taylor and Sasser, 1978).

$^d$ Percentage infected females determined by observing 30 females under a compound microscope for the presence or absence of mature endospores.
Table 4-2. Effect of different crops and *Meloidogyne arenaria* race 1 densities on mean second-stage juveniles (J2) per 100 cm$^3$ of soil, and galling and egg mass indices in the spring 2005 following a winter cover crop of hairy vetch.

<table>
<thead>
<tr>
<th>Nematode densities</th>
<th>J2$^a$</th>
<th>EM$^b$</th>
<th>GI$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (4 J2)</td>
<td>37.0 a</td>
<td>1.4 b</td>
<td>0.8 c</td>
</tr>
<tr>
<td>Med. (20 J2)</td>
<td>70.0 a</td>
<td>1.9 b</td>
<td>1.7 b</td>
</tr>
<tr>
<td>High (40 J2)</td>
<td>43.3 a</td>
<td>3.2 a</td>
<td>3.2 a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crops</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>44.5 a</td>
<td>1.6 b</td>
<td>1.4 b</td>
</tr>
<tr>
<td>Squash</td>
<td>50.1 a</td>
<td>2.8 a</td>
<td>2.4 a</td>
</tr>
</tbody>
</table>

Means are an average of six replications. Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Waller-Duncan $k$ ratio ($k=100$) $t$-test.

$^a$J2 per 100 cm$^3$ of soil extracted using the centrifugal-flotation technique (Jenkins, 1964).

$^b$Gall and egg mass index rating based on a 0-5 scale where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses, 2 = 3-10 galls or egg masses, 3 = 11-30 galls or egg masses, 4 = 31-100 galls or egg masses, and 5 = more than 100 galls or egg masses (Taylor and Sasser, 1978).
Table 4-3. Effect of crops and nematode inoculation densities on endospores per second-stage juvenile (J2) in the spring 2005 following a winter cover crop of hairy vetch.

<table>
<thead>
<tr>
<th>Nematode densities</th>
<th>Squash</th>
<th>Peanut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (4 J2)</td>
<td>1.6 c</td>
<td>3.5 b</td>
</tr>
<tr>
<td>Med. (20 J2)</td>
<td>2.2 b</td>
<td>2.7 c</td>
</tr>
<tr>
<td>High (40 J2)</td>
<td>4.2 a</td>
<td>4.3 a</td>
</tr>
</tbody>
</table>

Means are an average of six replications. Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ according to Waller-Duncan $k$ ratio ($k=100$) $t$-test.

$^a$ Number of endospores attached per J2 was calculated based on the first 20-J2 observed per sample.
Table 4-4. Effect of crops and nematode inoculation densities on endospores per second-stage juvenile (J2), and J2 per 100 cm³ of soil following an okra indicator crop, November 2005.

<table>
<thead>
<tr>
<th>Nematode densities</th>
<th>Endo/J2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>J2&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Squash</td>
<td>Peanut</td>
</tr>
<tr>
<td>Low (4 J2)</td>
<td>7.0 a</td>
<td>7.6 c</td>
</tr>
<tr>
<td>Med. (20 J2)</td>
<td>9.6 a</td>
<td>28.3 b</td>
</tr>
<tr>
<td>High (40 J2)</td>
<td>13.5 a</td>
<td>44.9 a</td>
</tr>
</tbody>
</table>

Means are average of six replications. Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ according to Waller-Duncan $k$ ratio ($k=100$) $t$-test.

<sup>a</sup> Number of endospores attached per J2 was counted based on the first 20 J2 observed per sample.

<sup>b</sup> J2 per 100 cm³ of soil extracted using the centrifugal-flotation technique (Jenkins, 1964).
Fig. 4-1. Effect of two crops and different nematode inoculations densities on endospores per second-stage juveniles (J2) after an okra crop, November 2005. Means are an average of six replications. Bars followed by the same lower case (squash) or upper case (peanut) letter are not significantly different at $P \leq 0.05$ according to Waller-Duncan $k$ ratio ($k=100$) $t$-test.
Fig. 4-2. Effect of two crops and different nematode inoculations densities on percentage infected females after an okra crop, November 2005. There were few or no galls on peanut plots hence data on percentage infected females was not available.
Table 4-5. Effect of different crops and nematode inoculation densities on mean gall (GI) and egg mass indices (EM) after an okra indicator crop, November 2005.

<table>
<thead>
<tr>
<th>Nematode densities</th>
<th>GI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EM&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (4 J2)</td>
<td>2.0 b</td>
<td>1.4 a b</td>
</tr>
<tr>
<td>Med. (20 J2)</td>
<td>2.7 a</td>
<td>1.5 a</td>
</tr>
<tr>
<td>High (40 J2)</td>
<td>2.7 a</td>
<td>0.9 b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crops</th>
<th>GI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EM&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squash</td>
<td>3.9 a</td>
<td>1.9 a</td>
</tr>
<tr>
<td>Peanut</td>
<td>1.0 b</td>
<td>0.6 b</td>
</tr>
</tbody>
</table>

Means are average of six replications. Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ according to Waller-Duncan $k$ ratio ($k=100$) $t$-test.

<sup>a</sup>Gall and egg mass index rated using a 0-5 scale where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses, 2 = 3-10 galls or egg masses, 3 = 11-30 galls or egg masses, 4 = 31-100 galls or egg masses, and 5 = more than 100 galls or egg masses (Taylor and Sasser, 1978).
Table 4-6. Effect of different crops and nematode inoculation densities on foliage fresh and dry weights, yield, and number of okra fruit, November 2005.

<table>
<thead>
<tr>
<th></th>
<th>Foliage</th>
<th>Root</th>
<th>Fruit wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FW (g)</td>
<td>DW (g)</td>
<td>FW (g)</td>
</tr>
<tr>
<td>Nematode densities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (4 J2)</td>
<td>187.3 a</td>
<td>22.6 a</td>
<td>33.5 a</td>
</tr>
<tr>
<td>Med. (20 J2)</td>
<td>172.7 a</td>
<td>19.7 a</td>
<td>29.7 a</td>
</tr>
<tr>
<td>High (40 J2)</td>
<td>180.5 a</td>
<td>21.8 a</td>
<td>29.6 a</td>
</tr>
<tr>
<td>Crops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>248.2 a</td>
<td>30.4 a</td>
<td>33.0 a</td>
</tr>
<tr>
<td>Squash</td>
<td>112.1 b</td>
<td>12.3 b</td>
<td>28.8 a</td>
</tr>
</tbody>
</table>

Means are average of six replications. Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ according to Waller-Duncan $k$ ratio ($k=100$) $t$-test.
Fig. 4-3. Number of endospore per second-stage juvenile (J2) across all seasons from spring 2003 to autumn 2005. Values are based on means of all plots. Data analyzed for individual seasons rather than across seasons.
Fig. 4-4. Number of second-stage juveniles (J2)/100 cm$^3$ of soil across all seasons from spring 2003 to autumn 2005. Values are based on means of all plots. Data analyzed for individual seasons rather than across seasons.
Peanut is one of the most important crops grown in the southern United States (Minton and Baujard, 1990). Most of the peanut in the United States are grown for peanut butter, peanut candy, roasted peanuts, peanut oil and other peanut products (Whitty, 2002). Production is concentrated in three major geographic regions of the United States; the southeast, Virginia and the Carolinas, and the southwest (Anonymous, 2005). The southern United States accounts for 64% of the total United States peanut production. The $4 billion United States peanut industry employs an estimated 150,000 people on-farm, shelling, and in manufacturing plants. The majority of the employment is concentrated in rural areas. Because of the concentration of the peanut industry and jobs in the rural communities of producing states, the economic impact of peanut is magnified in these regions (Bridges et al., 1994).

The peanut root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood is the most widespread and destructive nematode pathogen of peanut (Aerts and Nesheim, 2001; Dickson, 1985; Dickson and De Waele, 2005; Porter et al., 1984). In the United States, this nematode is the most aggressive species parasitizing peanut in Alabama, Florida, Georgia, and Texas, whereas it occurs less frequently in North Carolina, South Carolina and Virginia. In other regions of the world, *M. arenaria* is reported damaging peanut in Zimbabwe (Martin, 1958), Israel (Orion and Cohn, 1975), Egypt (Ibrahim and El-Saedy, 1976a), India (Dhurj and Vaishnav, 1981; Sakhuja and Sethi, 1985b; Sharma et al., 1978), Taiwan (Cheng and Tu, 1980; Cheng et al., 1981), and China (Zhang, 1985).
This species exists as two host races distinguished only on their basic reaction on peanut (Taylor and Sasser, 1978). Race 1 infects and reproduces on peanut, whereas race 2 does not. Both races are widespread in many regions where peanut is produced, and they both cause disease on numerous agronomic and horticultural crops. The presence of the two host races make the use of routine nematode advisory services difficult because of the time required to determine their identity (Dickson and De Waele, 2005). The only way they can currently be separated is by their reaction on peanut.

The current approaches to management of *M. arenaria* includes the use of nematicides (both fumigants and nonfumigants), cultural practices, biological control and resistant cultivars (Dickson and De Waele, 2005; Kinloch, 2003; Rich and Kinloch, 2005). These tactics are often used in an integrated manner. The soil fumigant 1,3-dichloropropene (1,3-D) has been shown to be the single most effective nematicide for managing root-knot and lesion nematodes in peanut infested fields in Florida (Dickson and De Waele, 2005; Rich and Kinloch, 2005). However, the use of nematicides has created great controversy because of associated environmental problems. 1,3-D is toxic to humans and animals (Anonymous, 1996) and as such, the US Environmental Protection Agency imposes worker protection guidelines and a 30-m buffer zone from any human dwellings (Telone II label, DowAgrosciences, Indianapolis, IN). Increased cost of nematicides also is a deterrent to their use by peanut growers.

One cultural practice important for managing nematode disease on peanut includes crop rotation. Rotating peanut with pasture grasses, namely bahiagrasses or bermudagrasses, is known to aid with root-knot nematode management (Rich and Kinloch, 2005). Development of resistance in peanut to root-knot nematodes is a recent
program that offers promise for the future as the source of resistance is incorporated into more commercially acceptable cultivars (Dickson and DeWaele, 2005; Simpson and Starr, 2001).

There are numerous biological agents including fungi and bacteria that parasitize or prey on root-knot nematode second-stage juveniles in soil (Chen and Dickson, 2005; Chen and Dickson, 2005). Among these agents one of the more promising is a bacterium, *Pasteuria penetrans* (Thorne) Sayre & Starr. This agent has been especially effective against the root-knot disease of peanut (Dickson et al., 1994; Chen and Dickson, 1998). *P. penetrans* has been shown to effectively suppress root-knot nematode populations in field and microplots experiments (Brown and Smart, 1985; Chen and Dickson, 1998; Chen et al., 1996b; Daudi et al., 1990; Dickson et al., 1994; Freitas et al., 2000, Oostendorp et al., 1991; Stirling, 1984; Trivino and Gowen, 1996; Weibelzahl-Fulton et al., 1996). Also, it is known to suppress *Meloidogyne* spp. on eggplant, tomato, wheat, tobacco, soybean, bean, pepper, hairy vetch, cucumber, peanut, rye, chickpea, kiwi, grape, brinjal, mung, and okra. Isolates of *Pasteuria* spp. have been reported to suppress *Heterodera avenae* and *H. zeae* on bermudagrass turf (Giblin-Davis, 1990), *H. elachista* on rice (Nishizawa, 1987), and *H. cajani* on cowpea (Singh and Dhawan, 1994).

To understand the role of this organism in causing soil suppressiveness the effect of different *P. penetrans* concentrations on attachment, penetration and fecundity were evaluated. Different attachment levels were achieved using different ratios of J2 to endospores. As the mean attachment level per juvenile increased the percentage of J2 penetrating host plant roots decreased. As low as 3.5 endospore/J2 reduced the ability of *M. arenaria* to enter host roots. Egg mass numbers were reduced by increasing numbers
of endospores attached per J2. The reduction in fecundity and J2 penetration are two ways *P. penetrans* induces soil suppressiveness. Field studies to determine whether *P. penetrans* can be transferred, established and managed in a new site on a large scale revealed that *P. penetrans*-induced suppressiveness can be transferred in the form of dried peanut roots, pegs and pods and okra roots. 1,3-D applications effectively reduced root-knot nematodes thereby decreases the build-up of *P. penetrans*, whereas chloropicrin applications prevented endospore development within developing root-knot nematodes thereby decreasing the build-up of *P. penetrans*. These affects were demonstrated by the fact that in fall 2005 after the final peanut harvest the greatest increase of *P. penetrans* was recorded in the untreated plots, demonstrating that the bacterium was increasing to suppressive densities in the nonfumigated plots. Microplots were used to determine the most effective crop and J2 density for amplification of *P. penetrans*. The long season peanut crop with a high density of J2 added was most effective for increasing densities of the bacterium as compared to short season crops, such as squash, and low densities of J2 added to microplots.
Table: A-1: The sequence of crops used during the transfer and management of *Pasteuria penetrans* and *Meloidogyne arenaria* race 1 from spring 2003 to autumn 2005 in a field experiment located at the Plant Science Unit, Citra, FL.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Crop</th>
<th>Effective dates</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring 2003</td>
<td>Peanut</td>
<td>April-September 2003</td>
<td></td>
</tr>
<tr>
<td>Autumn 2003</td>
<td>Wheat</td>
<td>October-March 2003</td>
<td>Amplification</td>
</tr>
<tr>
<td>Spring 2004</td>
<td>Peanut</td>
<td>April-September 2004</td>
<td></td>
</tr>
<tr>
<td>Autumn 2004</td>
<td>Vetch</td>
<td>October-March 2005</td>
<td>Management</td>
</tr>
<tr>
<td>Spring 2005</td>
<td>Peanut</td>
<td>April-September 2005</td>
<td></td>
</tr>
</tbody>
</table>
Table: A-2: The sequence of crops used for a microplot study located on campus, University of Florida, Gainesville, FL on the influence of host crops and different levels of *Meloidogyne arenaria* race 1 on amplification of *Pasteuria penetrans* from spring 2003 to autumn 2005.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Crop</th>
<th>Effective dates</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring 2003</td>
<td>Peanut/squash</td>
<td>April-September 2003</td>
<td>Establishment</td>
</tr>
<tr>
<td>Autumn 2003</td>
<td>Wheat</td>
<td>October-March 2003</td>
<td></td>
</tr>
<tr>
<td>Spring 2004</td>
<td>Peanut/squash</td>
<td>April-September 2004</td>
<td></td>
</tr>
<tr>
<td>Autumn 2004</td>
<td>Vetch</td>
<td>October-March 2005</td>
<td>Amplification</td>
</tr>
<tr>
<td>Spring 2005</td>
<td>Peanut</td>
<td>July 2005</td>
<td></td>
</tr>
<tr>
<td>Summer 2005</td>
<td>Okra</td>
<td>November 2005</td>
<td></td>
</tr>
</tbody>
</table>
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BIOGRAPHICAL SKETCH

George Muhia Kariuki was born in February 27, 1971, in Kiambu District of Central Kenya. He graduated with a Bachelor of Science degree in forestry, from Moi University, Kenya, in 1999. George received a Master of Science degree in plant pathology from the University of Nairobi, Kenya, working under the supervision of Prof. R. K. Mibey on the biological control of water hyacinth *Eichhornia crassipes* using fungi. He started the PhD program in nematology at the University of Florida, USA, in January 2003, working on the biological control of root-knot nematode *Pasteuria penetrans* under the supervision of Dr. Don W. Dickson. After graduation, he wants to work as a researcher in a well established research institution or a faculty member in a well-established university.