2015

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Investigations of *Pasteuria* and the Root-Lesion Nematode *Pratylenchus penetrans* in Soils
Collected from Certified Organic Farms in the Mid-Atlantic United States

Tiara Nicole King

Thesis submitted to the
Davis College of Agriculture, Natural Resources and Design
at West Virginia University

in partial fulfillment of the requirements for the degree of

Master of Science in
Plant and Soil Science
with an emphasis in Plant Pathology

James B. Kotcon, Ph.D., Chair
Louis M. McDonald, Ph.D.
Joseph B. Morton, Ph.D.
Alan J. Sexstone, Ph.D.

Division of Plant and Soil Sciences

Morgantown, West Virginia
2015

Keywords: bacterial parasite, biological control, certified organic, green bean, Mid-Atlantic, organic agriculture, organic farm survey, *Pasteuria*, *Phaseolus vulgaris*, plant-parasitic nematode, *Pratylenchus*, root lesion nematode, soil suppression

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ABSTRACT

Investigations of *Pasteuria* and the Root-Lesion Nematode *Pratylenchus penetrans* in Soils Collected from Certified Organic Farms in the Mid-Atlantic United States

Tiara Nicole King

Occurrence of lesion nematodes and *Pasteuria*, an obligate bacterial parasite, was surveyed in 34 soils collected from certified organic farms in the Mid-Atlantic United States. *Pasteuria* endospores were observed on *Pratylenchus crenatus* in six of 22 soils. Population density of *Pratylenchus* in soils lacking signs of *Pasteuria* correlated positively with soil pH, cation exchange capacity, and concentration of phosphorus pentoxide and calcium. *Pasteuria* was detected on *Pratylenchus* in nematode suppressive soils at the West Virginia University Organic Research Farm. Interactions between *Pasteuria* and *Pratylenchus penetrans* were studied in two laboratory bench-top experiments. Soil treatment effects on reproduction of *Pratylenchus penetrans* on green bean (*Phaseolus vulgaris*) cv. Jade in soils with *Pasteuria* were compared. Sterile and non-sterile Organic Research Farm soil treatments were planted with *Phaseolus vulgaris* cv. Jade and three of four treatments were inoculated with *Pratylenchus penetrans*. Mean population densities of *Pratylenchus* were significantly different among soil treatments ($P > 0.0001$) and *Pasteuria* endospores were observed on *Pratylenchus crenatus* from a control treatment. Twelve soils from the survey samples, six with and six without *Pasteuria* endospores detected on lesion nematodes, were used to compare eight soil treatment combinations in three randomized blocks. Treatment combinations consisted of soil type, time of harvest, presence of *Pasteuria*, and lesion nematode inoculation. Populations of *Pratylenchus penetrans* decreased in both *Pasteuria* soil treatments ($P > 0.03$). Population densities of *Pratylenchus* were not significantly different among soils with and without *Pasteuria* at the two-week harvest period ($P > 0.17$). At the 8-week harvest, lesion nematode population densities were significantly lower in soils with *Pasteuria* than in soils without *Pasteuria* ($P = 0.09$). Results suggest that reproduction of *Pratylenchus penetrans* on *Phaseolus vulgaris* cv. Jade is lower in soils with *Pasteuria* detected on lesion nematodes than in soils without *Pasteuria* detected on *Pratylenchus*.

Dedication

This work is dedicated to the memory of several family members, and especially to my dear friend Meredith Ann Thompson of Annandale, Virginia. Meredith was a fellow scientist, environmentalist, and nature aficionado. She would have been proud of this work and the lessons I learned. To my grandparents, John Robert King and Lois Virginia Woods King of Marion, Virginia, who brought unconditional love and patience to our family's table. To my great-grandmothers, Nannie Mae Reeves King, who modeled happiness as a choice, and Louise Virginia Thomas Woods, who taught me how to give loving hugs and pick a good switch off of a Forsythia bush. To my brother and sister, Mikal Wayne Deel, who made everyone near him feel loved, and Aimee Michelle Fisher, who was always an angel.

This work is also dedicated to all of my TIRELESS cheerleaders: family, friends, and colleagues; and especially to CE who showed me compassion, patience, and grace. To my siblings, Tiffany Leigh King-Hash, Robb Fisher, and Jenny Workman-Fisher. To my father, Michael Joseph Barrett, who teaches me to be kind and to value others based on their character. Most of all, this work is dedicated to my mother, Tonda Gail King-Barrett, for teaching me to be independent and introspective, and for being the greatest advocate of my efforts to live my dreams with passion and *tenacity*.

Acknowledgments

Financial support for this research was provided by the Organic Farming Research Foundation; The West Virginia University Davis College of Agriculture, Natural Resources and Design; and the USDA Hatch project NE-1040. Cultures of *Pratylenchus penetrans* were generously provided by the Nematology Laboratory at USDA-ARS Beltsville, MD. Soil analyses were conducted by the WVU Soil Chemistry Research Laboratory and the WVU Soil Testing Laboratory.

I pay deepest gratitude to my graduate advisor, Dr. James B. Kotcon, for his assistance, guidance and endless patience throughout this endeavor. His fervor and respect for science are unmatched and he has an unwavering ability to be positive and supportive at all times.

I sincerely appreciate the guidance, support, and feedback provided by my advisory committee members: Dr. Louis McDonald, Dr. Joseph Morton, and Dr. Alan Sexstone. Based on their unique areas of expertise, their recommendations provided me with a stronger foundation and a broader perspective for this research.

This work could not have been completed without the help of Joshua S. LaValle, Caroline A. Copenhaver, Angel Sampson, Evan Anderson, and Michelle Apolaro, all of whom reviewed sections of this report. Special thanks to Joshua and Caroline for their assistance with organizing the survey of organic farm soils, collecting and processing soil samples, and harvesting experiments.

I am thankful for the farm managers and growers who kindly participated in the survey of certified organic farms. It was a pleasure visiting your farms and getting to know you.

Additionally, I would like to thank and recognize the following individuals: Dr. Barton Baker for granting a teaching assistantship and extended funding during my tenure at WVU. Drs.

Gary K. Bissonnette and Barton Baker for funding to attend a Nematode-Bacteria Symbioses Workshop hosted by the University of Arizona. Dr. Daniel Panaccione for his support in preparation for seminar presentations and for inspiring my lifelong adoration of Mycology. Dr. William Bryan for his guidance during my teaching assistantship and many hours of engaging dialogue. Drs. Gary K. Bissonnette, William MacDonald, Mannon Gallegly, and Yong-Lak Park for enlightenment and entertainment during pastime conversations. I am also grateful to every member of the WVU faculty and staff for all they do to provide a positive and memorable experience for every student.

Finally, I would like to say thank you to every person who remained patient and supportive of me during this project.

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1.0 INTRODUCTION

Root-lesion nematode species in the genus *Pratylenchus* Filipjev are pathogenic to a wide variety of agricultural crops. *Pratylenchus penetrans* (Cobb) Filipjev and Shuurmans-Stekhoven is a particularly virulent species known to reduce crop yields and cause significant economic losses. Pest management options for lesion nematodes are limited, especially for organic crop production, which relies mainly on agricultural management practices and biological pest control. Several members of genus *Pasteuria* Metchnikoff are naturally occurring bacteria that parasitize plant-parasitic nematodes. In the United States, formulations of *Pasteuria* species are used to control populations of root-knot nematodes (*Meloidogyne* spp.) and soybean cyst nematodes (*Heterodera glycines*). There are currently no commercially available formulations of *Pasteuria* to treat soils infested with *Pratylenchus penetrans*.

New isolates of *Pasteuria* are obtained from infected nematodes recovered from soils collected for nematode surveys. Few observations of lesion nematodes infected by *Pasteuria* have been reported (Chen and Dickson, 1998; Gonzaga and Santos, 2009). The geographic distribution and frequency of *Pasteuria* and *Pratylenchus* species occurring in organic cropping soils in the Mid-Atlantic United States currently is not known. *Pasteuria* endospores were observed on *Pratylenchus* from vegetable plot soils at the West Virginia University Organic Research Farm (J. Kotcon, pers. commun.). Lesion nematode populations fail to increase in the presence of suitable host crops and the source of nematode suppression in these soils has not been investigated. Interactions of *Pasteuria* and lesion nematodes in organically managed soils have yet to be measured.

The objective for this study was to increase the understanding of interactions between *Pasteuria* and *Pratylenchus penetrans* and to determine the role *Pasteuria* species may have as a

biological control agent of lesion nematode populations in agricultural crop production systems. Therefore, a survey was conducted to establish the occurrence of *Pasteuria* on lesion nematodes in certified organic soils collected from Maryland, Ohio, Pennsylvania, Virginia, and West Virginia. Soil samples collected for this survey that met specific criteria were planted with corn (*Zea mays*) and green bean (*Phaseolus vulgaris*) to increase detection of *Pasteuria* on the natural lesion nematode populations in these soils.

Experiments were conducted to determine effects of *Pasteuria* on reproduction of *Pr. penetrans* on green bean (*Phaseolus vulgaris*) cv. Jade in certified organic soils. One experiment was designed to investigate the source of nematode suppression in WVU Organic Research Farm soils and evaluate effects of soil volume on reproduction of *Pr. penetrans* on green bean. Twelve of the soils collected during the survey of certified organic farms were used in another experiment that compared reproduction of *Pr. penetrans* in soils with and without *Pasteuria*.

2.0 LITERATURE REVIEW

2.1 Biology of *Pasteuria* Metchnikoff, 1888

Members of genus *Pasteuria* are gram-positive, endospore-forming, obligate parasites of cladocerans and nematodes. Isolates of *Pasteuria* are associated with more than 300 species of nematodes, and many isolates are reported to suppress populations of plant-parasitic nematodes in cropped soils (Chen and Dickson, 1998). Investigations of the biological control potential of these naturally occurring nematode antagonists are underway globally.

2.1.1 Taxonomy

In 1888, Metchnikoff described *Pasteuria ramosa* as a parasite of water fleas, *Daphnia* spp. (Ebert et al., 1996). In 1906, Cobb observed the nematode *Dorylaimus bulbiferous* infected with refractile endospores and suggested the parasite was a sporozoan. When Thorne observed

similar endospores on *Pratylenchus brachyurus* in 1940, he classified it as *Duboscqia penetrans* (Sayre et al., 1988). Several decades later, scanning electron micrographs of *D. penetrans* revealed morphological characteristics similar to that of *Bacillus* species and it was renamed *Bacillus penetrans* (Mankau, 1975). Sayre and Starr (1985) reported definitive evidence that *B. penetrans* was distinctly dissimilar from members of genus *Bacillus* and more closely resembled the cladoceran parasite *Pasteuria ramosa* as originally described by Metchnikoff. They suggested that *B. penetrans* was related to *Pa. ramosa* and proposed the name *Pasteuria penetrans* (Thorne) to replace *B. penetrans* Mankau 1975.

Similarities in the ultrastructure and development of *Pasteuria* species and 16S rRNA sequence data indicate nematode-parasitic *Pasteuria* species and *Pa. ramosa* are closely related, which supports grouping of these organisms in the same genus (Mankau and Imbriani, 1975; Sayre et al., 1979, 1983, 1991a, 1991b; Sayre and Starr, 1985; Starr and Sayre, 1988; Sturhan et al., 1994, 2005; Anderson et al., 1999; Bekal et al., 2001; Giblin-Davis et al., 2001, 2011; Preston et al., 2003; Atibalentja et al., 2000, 2004a, 2004b; Charles et al., 2005; Schmidt et al., 2008, 2010). In 2009, the genus *Pasteuria* was removed from Family *Alicyclobacillaceae* and reclassified into Family *Pasteuriaceae*, Order *Bacillales* (Fritze, 2004; Garrity et al., 2005; Dickson et al., 2009).

With regard to early scientific literature, *Duboscqia penetrans*, *Bacillus penetrans*, and *Pasteuria penetrans* may refer to isolates or species of nematode-parasitic *Pasteuria* and are accurately referred to as *Pasteuria* species, or as “members of the *Pasteuria penetrans* group” (Sayre and Starr, 1985; Sayre et al., 1991b; Chen and Dickson, 1998). Some *Pasteuria* species referred to as *Pasteuria penetrans* may represent another isolate that has since been reclassified.

Pa. penetrans is accepted as the species that is primarily parasitic on members of *Meloidogyne* and *Pa. penetrans sensu stricto* are parasites of *Meloidogyne incognita* (Starr and Sayre, 1988).

2.1.2 *Pasteuria* species diversity

Pasteuria species have been traditionally distinguished by host preference, life cycle, and morphology of vegetative structures. The type species *Pasteuria ramosa* Metchnikoff 1888 is a parasite of water fleas (*Cladocera*; *Daphnidae*) (Sayre et al., 1979; Ebert et al., 1996). Metchnikoff observed *Pa. ramosa* on *Daphnia pulex* Leydig and *D. magna* Strauss. In the century following Metchnikoff's report, researchers described a variety of cladoceran parasites similar to *Pa. ramosa* and debated the taxonomic classification of these organisms (Sayre et al., 1983). Sayre and Wergin (1977) and Sayre et al. (1983) compared ultrastructure and morphology of *Pa. ramosa* and the nematode-parasitic species "*Bacillus penetrans*" Mankau. These authors reviewed the taxonomic history of *Pa. ramosa* and clarified classification of the organism as the parasite of cladocerans originally described by Metchnikoff.

Most known isolates of *Pasteuria* are parasites of nematodes. *Pasteuria penetrans* parasitize *Meloidogyne*, *Pa. thornei* are parasites of *Pratylenchus*, and *Pa. nishizawae* are parasites of *Heterodera* and *Globodera* (Sayre and Starr, 1985; Starr and Sayre, 1988; Sayre et al., 1991a, 1991b). *Candidatus* species include *Pasteuria usgae* on *Belonolaimus longicaudatus*, *Pa. hartismeri* on *Meloidogyne ardenensis*, and *Pa. aldrichii* on *Bursilla* (Giblin-Davis et al., 2003, 2011; Bishop et al., 2007). Sturhan et al. (1994), Orajay (2009), and Schmidt et al. (2010) describe isolates of *Pasteuria* species infecting *Heterodera goettingiana*, *Mesocriconema ornatum*, and *Rotylenchulus reniformis*.

Chen and Dickson (1998) stated that while most reports of *Pasteuria*-like organisms are based on observation of endospore attachment, this does not confirm infection of the nematode

host. Host specificity and endospore attachment reflect “host preference.” A few *Pasteuria* species infect multiple nematode genera; however, most species have a narrow host range and this host preference is used, in part, to categorize isolates of *Pasteuria*.

2.1.3 Life cycle and mode of infection

Pasteuria are pleomorphic and varying descriptions of the life cycle, ultrastructure, and morphology of nematode-parasitic *Pasteuria* species have been reported (Mankau, 1975; Mankau and Imbriani, 1975; Imbriani and Mankau, 1977; Mankau and Prasad, 1977; Sayre and Wergin, 1977; Sayre and Starr, 1985, 1988; Sayre et al., 1979, 1983, 1988, 1991a, 1991b; Starr and Sayre, 1988; Sturhan et al., 1994, 2005; Chen et al., 1997; Serracin et al. 1997; Chen and Dickson, 1998; Giblin-Davis et al., 2001, 2011; Preston et al., 2003; Atibalentja et al., 2004a; Davies, 2009; Schmidt et al., 2010; Davies et al., 2011). The majority of these reports were summarized by Dickson et al. (2009).

The host-parasite interactions of *Meloidogyne* and *Pasteuria penetrans* have been studied extensively and are the model used to describe the life cycle and development of *Pasteuria* on plant-parasitic nematodes (Dickson et al., 2009). Davies et al. (2011) proposed amendments to the life cycle based on electron micrographs of *Pasteuria*-infected *Meloidogyne* females and juveniles. Authors described three phases of the cycle: Phase I, attachment and germination; Phase II, rhizoid formation and exponential growth; and Phase III, sporogenesis. Proliferation of *Pasteuria* throughout the nematode body eventually disrupts metabolic processes and results in nematode death.

During Phase I, root-knot nematode larvae (J2) move through soil where mature *Pasteuria* endospores attach to the cuticle of the nematode. Collagen-like fibers on the endospore surface may play a role in endospore attachment (Davies, 2009). Although many endospores

may attach to a nematode, only one is needed for infection to occur. One to 20 endospores have been observed attached to root-knot nematode juveniles. The J2 enters a plant root and the endospore germinates before the J2 molts into a third-stage juvenile. Germination occurs within 4 to 12 days after a root-knot nematode J2 establishes a feeding site in a host root (Sayre and Wergin, 1977; Davies et al., 1988). Germination begins when a germ tube emerges from the endospore core and penetrates the cuticle and hypodermis of the nematode body. Mankau and Imbriani (1975) and Mankau et al. (1976) suggested enzymes aid in germination. Davies et al. (1990) observed mature *Pasteuria* endospores in root-knot nematode J2s and suggested that establishment of a feeding site is not required for germination to occur.

Phase II begins when the germ tube differentiates into either a filamentous, rhizoid-like structure or microcolonies of rod-shaped structures that appear to be similar to *Bacillus* cells. The rhizoid-like structure grows into the pseudocoelom until filamentous growth discontinues, and then microcolonies develop. When filamentous growth does not occur, the microcolonies develop inside the body cavity at the site of penetration. The rod-shaped cells reproduce quickly and form tight clusters that have a granular appearance during this period of exponential growth. Rod-shaped cells were observed by Mankau (1975) in *M. javanica* infected by *Pa. penetrans*; however, these structures were not verified to be cells of *Pasteuria*.

In Phase III, there is a transition from exponential to stationary growth and sporogenesis occurs. The rod-shaped cells thicken and form thalloid-like chains that break apart from the granular masses. Cytoplasmic changes occur within each cell as it develops into an ovate sporangium. A membrane develops inside the sporangium, and the upper part of the cell — the forespore — is separated from the lower parasporal section. The cytoplasm within the forespore contracts into an electron-opaque structure that becomes the endospore. In the parasporal section,

fibers form around the outer wall of the endospore, and a loose outer membrane — the exosporium — develops around these parasporal fibers. The remaining portion of the paraspore degenerates and each sporangium encloses a single mature endospore. Sporangia fill the nematode body cavity and endospores are released into the soil when the nematode cadaver degrades.

Previous reports of the life cycle, ultrastructure, and morphological characteristics of *Pa. penetrans* and other *Pasteuria* species differ from Davies et al. (2011). Serracin et al. (1997) described five stages of *Pa. penetrans* development in *M. arenaria*: (i) endospore germination, (ii) vegetative growth, (iii) thalli differentiation, (iv) sporulation, and (v) maturity of endospores. Authors presented photographs of structures observed with light microscopy that were verified by scanning electron microscopy. Endospore attachment and germination, as well as final stages of endospore formation, are similar to that of Davies et al. (2011). However, developmental stages between germination and sporulation were described as mycelial and not bacterial. Similar descriptions of *Pa. penetrans* have been presented by other authors that were previously noted in this section.

After endospore germination, the germ peg develops into dichotomously branched, septate mycelia that contain hyphal cells. Septate hyphal cells have a compound cell wall with inner and outer membranes, and septa are formed by the inner membrane. Lysis of intercalary cells forms daughter colonies of mycelia that are passively transported throughout the nematode body. Sporulation begins when distal cells of the hyphae bifurcate, swell into ovate cells, and develop into ovate sporangia that lyse from parent hyphae. Hyphae cease growth and eventually disintegrate. Observations of concurrent vegetative growth and sporulation in *M. javanica* have been reported (Mankau and Imbriani, 1975).

Mycelial vegetative growth was also reported for *Pasteuria* on *Pratylenchus*, *Heterodera*, *Belonolaimus*, *Anaplectus*, *Plectus*, and *Bursilla* by Sayre et al. (1988, 1991a, 1991b), Starr and Sayre (1988), Sturhan et al. (1994, 2005), Giblin-Davis et al. (2001, 2011), and Atibalentja et al. (2004a). Dickson et al. (2009) indicated this mycelial-like development is only observed in species of *Pasteuria* and *Thermoactinomyces*. Future reports of the *Pasteuria* life cycle may propose amendments that include the stages described by Davies et al. (2011).

While *Pasteuria* infection can occur in all life stages of vermiform and sedentary nematodes, no infection has been reported in eggs. Infected juveniles have been observed in several nematode species and nematode feeding is not required for endospore germination to occur (Chen and Dickson, 1998; Bird et al., 2003; Davies, 2009; Hewlett et al., 2010; Schmidt et al., 2010; Davies et al., 2011). Factors that affect endospore attachment and germination include species of *Pasteuria*, species of host nematode, soil texture, temperature, moisture, and pH (Serracin et al., 1997; Chen and Dickson, 1998; Mateille et al., 2002; Dabiré and Mateille, 2004).

2.1.4 Sporangia and endospores

The morphology of *Pasteuria* endospores differs from that of other endospore-forming bacteria. *Pasteuria* has two morphological types of endospores. One form is enclosed in a loose membrane called the exosporium. The other form is missing the exosporium and has two recognizable parts: (i) a central, round endospore encased by (ii) a material referred to as the “peripheral matrix” or “perisporium” (Dickson et al., 2009). This peripheral matrix is made up of microfibrillar strands called parasporal fibers. These fibers, which are approximately 1.5 nm thick, spread out and down the sides of the endospore toward the nematode cuticle. Endospore morphometrics are difficult to assess due to the amorphous nature of the parasporal fibers.

The morphology of sporangia and endospores varies within and between isolates of *Pasteuria*. The cladoceran parasite, *Pa. ramosa*, has teardrop-shaped sporangia that are 3.3 to 4.1 μm in diameter and 4.8 to 5.7 μm in height, and endospore diameter ranges from 2.1 to 2.4 μm (Starr and Sayre, 1988; Sayre et al. 1991b). Sayre et al. (1991b) determined sporangia and endospore morphometrics for *Pa. penetrans*, *Pa. nishizawae*, and *Pa. thornei* with light microscopy. *Pa. penetrans* sporangia are $4.5 \pm 0.3 \mu\text{m} \times 3.6 \pm 0.3 \mu\text{m}$ (diameter and height), and endospores are $2.1 \pm 0.2 \times 1.7 \pm 0.2 \mu\text{m}$. Sporangia of *Pa. nishizawae* are $5.3 \pm 0.3 \mu\text{m} \times 4.3 \pm 0.3 \mu\text{m}$ and endospores are $2.1 \pm 0.2 \times 1.7 \pm 0.1 \mu\text{m}$. *Pa. thornei* sporangia are rhomboid-shaped with a diameter of $3.5 \pm 0.2 \mu\text{m}$ and height of $3.1 \pm 0.2 \mu\text{m}$, and endospores are $1.6 \pm 0.1 \mu\text{m} \times 1.5 \pm 0.1 \mu\text{m}$. Sporangia of *Pa. penetrans* and *Pa. nishizawae* are cup-shaped.

Giblin-Davis et al. (1990) described spores from several plant-parasitic nematodes in Bermudagrass. They reported small and large endospore isolates of *Pasteuria* on *Hoplolaimus galeatus*, with sporangia diameter ranging from 3.19 to 4.64 μm and width of endospores ranging from 1.45 to 2.45 μm for the small endospore isolate, versus 6.53 to 8.05 μm and 2.72 to 3.54 μm , respectively, for the large endospore isolate. For *Pasteuria* observed on *Belonolaimus longicaudatus*, sporangia diameter ranged from 4.71 to 7.00 μm and endospore width varied from 2.33 to 3.78 μm . Sporangia for *Pasteuria* observed on *Tylenchorhynchus annulatus* were 3.98 to 5.38 μm in diameter and width of endospores was 2.21 to 2.95 μm . The diameter of sporangia for *Pasteuria* on *Helicotylenchus microlobus* was 2.78 to 4.73 μm and endospore width was 1.39 to 2.50 μm . For *Pa. penetrans* observed on *Meloidogyne* juveniles, sporangia ranged from 2.91 to 4.19 μm in diameter and endospore width was 1.22 to 1.98 μm .

An adult root-knot nematode female infected with *Pa. penetrans* may be filled with 2×10^6 endospores (Sayre et al., 1991b; Dickson et al., 2009). Sayre et al. (1991b) stated that the

number of sporangia in *Meloidogyne* females was four times that of *Heterodera* females and also reported “a few hundred sporangia” present in lesion nematode cadavers. Atibalentja et al. (1998) reported 3×10^5 endospores from *Heterodera glycines* females infected with an isolate of *Pasteuria* from Illinois. Giblin-Davis et al. (2001) reported the average number of endospores recovered from *B. longicaudatus* cadavers was $1,483 \pm 1,199$ for females, $3,633 \pm 3,095$ for males, and $4,700 \pm 1,972$ for juveniles.

Pasteuria endospores are resistant to heat, desiccation, and some nematicides (Stirling et al., 1986; Chen and Dickson, 1998; Bekal et al., 2000; Freitas et al., 2000; Kariuki and Dickson, 2007). Bekal et al. (2000) reported that *Pa. penetrans* endospores in a soil sample that had been dried and stored for 21 years were infective to *M. incognita*. Juveniles were inoculated into moistened soil, and endospore-attached nematodes were recovered. *M. incognita* were cultured on tomato at 26°C in this soil, and infected female nematodes were observed after 8 weeks.

Attachment of *Pa. penetrans* endospores to *Meloidogyne* juveniles in soil is affected by nematode motility in soil, as well as soil temperature and moisture (Brown and Smart, 1984; Stirling et al., 1986; Oostendorp et al. 1990; Hatz and Dickson, 1992). Brown and Smart (1984) observed significantly higher proportions of *M. incognita* juveniles attached with *Pa. penetrans* endospores from soils that were moistened 3 days prior to nematode inoculation compared to soils moistened the day of inoculation ($P < 0.001$). Stirling et al. (1990) reported that attachment of *Pa. penetrans* endospores to *M. javanica* juveniles was higher at 27°C than at 18°C.

2.1.5 Use of *Pasteuria* for biological control of plant-parasitic nematodes

The study of *Pasteuria* as biological control agents of plant-parasitic nematodes is time-intensive and laborious. Initial efforts to produce *in vitro* cultures of *Pa. penetrans* were thwarted by obligate parasitism and strict host specificity. Therefore, researchers developed various *in*

vivo methods to culture *Pa. penetrans* on host nematodes, but these methods are not practical for commercial-scale production (Mankau, 1975; Mankau and Prasad, 1977; Dutky and Sayre, 1978; Stirling and Wachtel, 1980; Sharma and Stirling, 1991; Hewlett and Dickson, 1993; Chen et al., 1996a, 1996b; Hewlett et al., 2004).

2.1.5.1 Methods of endospore inocula production

Mankau (1975) and Mankau and Prasad (1977) propagated endospore-infested soils from a naturally occurring background population of *Pa. penetrans* discovered in *M. javanica* pot cultures. This population of *M. javanica* and *Pa. penetrans* had been collected from fields near Riverside, CA. Infected *Meloidogyne* populations were replanted with tomato seedlings and inoculated with healthy juveniles at 6-month intervals. The amount of endospore inoculum was not determined. Instead, soil samples were assayed to determine the level of observed infection. When at least 50% of *Meloidogyne* J2s inoculated in a sample became infected after exposure to the soil, it was considered an endospore-infested soil. These soils were periodically air-dried at 25 to 30°C and then stored for future use.

Methods for *in vivo* endospore production were optimized by Stirling and Wachtel (1980) and by Sharma and Stirling (1991), among others. Infected *M. javanica* females were dissected from tomato roots and crushed in water; then concentrations of *Pa. penetrans* endospores were determined with a hemacytometer. Stirling and Wachtel (1980) reported one to ten endospores attached to *M. javanica* juveniles that soaked in a suspension of 500,000 to 3,000,000 *Pa. penetrans* endospores/mL for 24 hours. Endospore-encumbered juveniles were inoculated on tomato in greenhouse pot culture (5,000 J2 per plant). After 8 weeks at 27°C, tomato roots were harvested and dried, then pulverized until the material passed through a 710- μ m-pore sieve. This root material was either combined with soil or used to prepare liquid

endospore suspensions. Both endospore formulations were used to conduct attachment assays and to amplify *Pa. penetrans* in greenhouse pot culture.

In one assay, the percent of endospore-attached juveniles recovered 24 hours after inoculation into endospore-infested soil was 39%, 45%, 85%, and 99% for 10, 40, 70, and 100 mg root material per kg soil, respectively. Juveniles had one to six endospores attached. In another assay, a suspension containing 0.2 grams of endospore-infested material was ground with a mortar and pestle and root debris was removed with a 25- μ m-pore sieve. Juvenile nematodes agitated in 100 mL of this endospore suspension using an aquatic air bubbling system had endospores attached after 5 hours.

Sharma and Stirling (1991) compared production of *Pa. penetrans* endospores on *M. javanica* grown on tomato (*Solanum lycopersicum* cv. Floradade or cv. Tiny Tim) in two types of planting containers. Tomato seedlings in 15-cm-diameter pots were inoculated with 5,000 J2s and seedlings in 34 X 28 X 4.5-cm trays were inoculated with 100,000 J2s. Juveniles had 5 to 20 endospores attached at the time of inoculation. Plants were grown in a greenhouse at 20 to 30°C and harvested 60 to 90 days after nematodes were inoculated. Roots were shaken to remove sand, then air-dried and milled into a powder. The material was soaked in an enzyme solution for 3 hours, then ground in a mortar and pestle for 5 minutes. The suspension was diluted with water and endospores were quantified with a hemacytometer. The amount of endospore-filled tomato root material produced in the trays was five times greater than in the pots. Ten to 24 grams of tomato root material collected from trays contained 10-120 X 10⁸ endospores. In their study, the primary factors that affected endospore production were plant species, density of nematode inoculum and time of harvest.

These methods of producing dry and liquid forms of *in vivo*-produced *Pa. penetrans* endospores enabled further laboratory and field studies of *Pasteuria* on *Meloidogyne*. After reliable means of producing *in vivo* inocula were developed, research focused on increasing the amount of endospore inocula, improving endospore quantification, and reducing the amount of time needed to conduct experiments.

Using methods described above to produce *Pa. penetrans* isolate P-100 root powder inoculum, Hewlett and Dickson (1993) developed a centrifugation endospore attachment procedure. A 200- μ L suspension of endospores and *M. javanica* J2s were combined in a 250- μ L microfuge tube and centrifuged at 9,500g for 2 minutes. At 1,000 endospores/200 J2, 61% of juveniles had 0.5 to 1.5 endospores/nematode attached. At 10,000 and 100,000 endospores/200 J2, 100% of juveniles had 4.4 to 7.7 attached endospores/J2 and 21.8 to 37.2 attached endospores/J2, respectively. This method decreased the amount of time needed to conduct endospore attachment assays.

Pasteuria endospores may be difficult to distinguish from *Bacillus* endospores, fungal spores and particles of debris in suspension. Chen et al. (1996a) questioned early assessments of endospore concentrations. These authors compared six endospore quantification methods and reported highest precision and accuracy with mortar disruption and machine disruption. *Pa. penetrans* isolate P-20 was propagated on *M. arenaria* race 1 grown on tomato (*Solanum lycopersicum* cv. Rutgers) in greenhouse pot culture. Endospore-infested root material was milled until it passed through a 5-mm-pore sieve. For mortar disruption, a 0.5-gram subsample was mixed with 5 mL deionized water, ground with a mortar and pestle, and then diluted up to 100 mL in an Erlenmeyer flask. For machine disruption, roots were milled until the material passed through a 250- μ m-pore sieve and a 0.5-gram subsample was mixed into 100 mL

deionized water. Endospore concentrations were 83.7 million and 79 million endospores per gram of root for mortar disruption and machine disruption, respectively. The least amount of debris was observed in suspensions prepared by machine disruption, and this method allowed for precise and accurate assessment of endospore concentrations.

Continued development and modifications of these methods support investigations of *Pasteuria* as a biological control for root knot nematodes in field and laboratory assays. Researchers are interested in finding *Pasteuria* isolates that parasitize multiple nematode hosts. Many host range studies have been conducted, as have studies of disease suppressive soils in which *Pasteuria* has been observed on nematodes of interest.

2.1.5.2 Suppression of *Meloidogyne* by *Pasteuria*

The potential use of *Pasteuria penetrans* for biological control of *Meloidogyne* has been investigated by several authors. Some studies induced suppression of root-knot nematodes by incorporating *in vivo*-produced endospore inocula, and others compared treatments of soils observed to be naturally suppressive to root-knot nematodes. Suppression of *Meloidogyne arenaria*, *M. incognita* and *M. javanica* by *Pa. penetrans* has been reported for several field soils (Oostendorp et al., 1991a; Chen et al., 1994; Chen et al., 1996b; Weibelzahl-Fulton et al., 1996; Freitas et al., 2000; Cetintas and Dickson, 2004; Kariuki and Dickson, 2007).

Oostendorp et al. (1991a) conducted a 2-year field microplot experiment to determine effects of *Pa. penetrans* isolate P-20 on infection of *M. arenaria* and peanut yield. There were three treatments: (i) *M. arenaria* and *Pa. penetrans* present, (ii) *M. arenaria* only, and (iii) untreated control. *Pa. penetrans* isolate P-20 was originally collected from *M. arenaria* race 1 found in a peanut field naturally infested with *Pasteuria* in Levy County, Florida. Greenhouse cultures of this isolate were maintained on *M. arenaria* race 1 on tomato (*Lycopersicon*

esculentum Mill. cv. Rutgers). Endospore-filled *M. arenaria* females were dissected from infected peanut hulls and crushed in water. Juveniles were incubated in the suspension on a shaker and after 3 hours, 80% of the J2s had one or more endospores attached. Sixty tomato plants were each inoculated with 1,000 J2s, with 30 of the plants receiving endospore-attached juveniles. Thirty tomato plants for the untreated control were not inoculated. Plants grew for 85 days before roots were harvested and mixed with corresponding soil. The tomato root and soil inocula were transferred to the microplots and peanuts were direct seeded into each plot in late spring each year.

Peanut yields from the untreated control plots were significantly higher than other treatments over all seasons. After two growing seasons, peanut yields were significantly higher in treatments with *Pa. penetrans* on *M. arenaria* than in treatments with *M. arenaria* alone ($P \leq 0.05$). Initial and final data indicated the percent of juveniles recovered from *Pa. penetrans* treatments that had one to four endospores attached increased from 7% to 28%, and juveniles with five or more endospores attached increased from zero to 41.6%. This study showed that *in vivo* produced *Pasteuria* inoculum can increase on host nematodes in field microplots.

Chen et al. (1996b) compared inoculum densities of 0, 1,000, 3,000, 10,000, and 100,000 *Pa. penetrans* (isolate P-20) endospores per gram soil in a 2-year field study of *M. arenaria* on peanut (*Arachis hypogaea*). The methods described in the previous section were used to prepare endospore-infested tomato root material. Clay flue liner microplots (25-cm X 40-cm X 100-cm) were imbedded in field soils and populations of *M. arenaria* were introduced and augmented on tomato (*Solanum lycopersicum* cv. Rutgers). For each microplot, a suspension of endospore-infested root powder was thoroughly mixed into the top 20-cm of soil and a peanut seedling (*A. hypogaea* cv. Florunner) was planted. Signs and symptoms of root-knot parasitism on peanut were

significantly reduced at inoculum densities of 10,000 and 100,000 *Pa. penetrans* endospores per gram soil. At 100,000 endospores per gram soil, peanut yields increased by 94% and indices for pod and root galls were reduced by 90% and 81%. The minimum inoculum dose required to suppress *M. arenaria* on peanut was 10,000 endospores per gram soil.

Chen et al. (1994) noted decreased symptoms of nematode damage in a monocropped tobacco field that contained a mixed population of *M. incognita* and *M. javanica*. *Pa. penetrans* had been reported on *Meloidogyne* juveniles recovered from these soils. Weibelzahl-Fulton et al. (1996) presented evidence to support the hypothesis that *Pa. penetrans* was the cause of root-knot nematode suppression in these soils.

Freitas et al. (2000) compared effects of soil fumigants on *Pa. penetrans* infecting *M. arenaria* on tomato and tobacco in field and greenhouse trials. Combinations of chloropicrin with 1,3-D or methyl bromide reduced infectivity of *Pa. penetrans* to *M. arenaria*, but metam sodium alone did not inhibit infection. Results also indicated that the naturally occurring suppression of *M. arenaria* populations in field soils was caused by *Pa. penetrans*.

Cetintas and Dickson (2004) evaluated suppression of *M. arenaria* by *Pa. penetrans* in a monocropped peanut field over a 4-year period. Although percentages of endospore-attached juveniles and infected females recovered from *Pa. penetrans*-infested soils were higher than those from control treatments, differences in peanut yields were not significant. *M. javanica* was discovered in experimental plots and a laboratory assay confirmed that *Pa. penetrans* isolate P-20, which is infective to *M. arenaria*, did not infect *M. javanica*. Infestation of field plots by *M. javanica* interfered with detection of suppressive effects of *Pa. penetrans* to *M. arenaria*. This explanation was supported by a laboratory assay that eliminated background nematode populations in four field soil treatments: (i) autoclaving, (ii) microwaving, (iii) air-drying, and

(iv) untreated. Results indicated that *Pa. penetrans* endospores were killed by the autoclave treatment and *M. arenaria* was suppressed by *Pa. penetrans* in the other three treatments.

Kariuki and Dickson (2007) established a population of *Pa. penetrans* on *M. arenaria* at a new field site by incorporating infested plant material into the soil. Okra roots and peanut galls, pods, and roots that were infested with *Pa. penetrans* were dried and then tilled into the soil. *M. arenaria*-infested plant material was then incorporated into these soils. Peanut and winter wheat were cultivated in soils to augment populations of *Pa. penetrans* and *M. arenaria*. At plant harvest, infection of *M. arenaria* by *Pa. penetrans* was confirmed based on observations of endospore-attached juveniles and infected females collected from soil and root assays. Field trials conducted at this site confirmed suppression of *M. arenaria* by *Pa. penetrans*.

2.1.5.3 Use of *Pasteuria* for biocontrol of other nematode species

Isolates of *Pasteuria* have been reported for cyst nematodes, *Heterodera* and *Globodera*, and some are suppressive to cyst nematode populations (Nishizawa, 1984, 1986, 1987; Davies et al., 1990; Sayre et al., 1991a, 1991b; Noel and Stanger, 1994; Sturhan et al., 1994; Atibalentja et al., 1998, 2000, 2004a; Ma et al., 2005; Noel et al., 2010). Sayre et al. (1991b) compared the morphology and host range of *Pasteuria* isolates reported by Nishizawa (1986, 1987) on *Heterodera elachista* and *H. glycines* and defined these isolates as *Pasteuria nishizawae*. Noel and Stanger (1994) reported a *Pasteuria* isolate on *H. glycines* recovered from research plots at USDA Urbana, Illinois. The source of this isolate is unknown and authors indicated the morphology of this isolate was not similar to *Pa. nishizawae*.

Atibalentja et al. (1998) assessed population dynamics of *H. glycines* and its associated *Pasteuria* isolate originally reported by Noel and Stanger. Analyses of the percentages of juveniles attached with endospores, the numbers of endospores attached per J2, and nematode

density indicated that *Pasteuria* was maintaining nematode populations below the damage threshold. This report indicates that naturally occurring populations of *Pasteuria* are capable of suppressing *H. glycines* in field soils.

In a 7-year field trial, Noel et al. (2010) established suppression of soybean cyst nematodes by incorporating *Pa. nishizawae*-infested soil into microplots that were naturally infested with *Heterodera glycines*. Eight liters of *Pa. nishizawae*-infested soil were plowed into a 12-m-long row of *H. glycines*-infested soil. Nematode densities were significantly lower in samples that had *Pa. nishizawae*-infested soil added than in plots without *Pa. nishizawae* ($P \leq 0.05$). This study determined that suppression of *H. glycines* by *Pa. nishizawae* can be transferred.

A few reports of *Pasteuria* on *Pratylenchus* indicated infection of *Pr. brachyurus* and *Pr. scribneri* by *Pa. thornei*; however, reports of *Pasteuria* species suppressing *Pr. penetrans* are not present in current literature. The first report of *Pasteuria* observed on a plant-parasitic nematode was *Pasteuria thornei* on *Pratylenchus brachyurus* (Thorne, 1940; Sayre et al. 1988). Thorne (1940) described a parasite of *Pratylenchus pratensis* and classified it as *Duboscqia penetrans*. When Sayre et al. (1988) studied Thorne's original preserved specimens, they noted that specimens had been relabeled to indicate the host nematode was *Pr. brachyurus* and not *Pr. pratensis*.

Starr and Sayre (1988) established *Pasteuria thornei* as the species of *Pasteuria* parasitic to root-lesion nematodes and indicated *Pratylenchus brachyurus* was its primary host. Future taxonomic studies may differentiate isolates that parasitize lesion nematodes other than *Pr. brachyurus*. Most studies of *Pasteuria* on lesion nematodes specify infection of *Pr. brachyurus*

and *Pr. scribneri* (Mankau, 1975; Mankau and Prasad, 1977; Dutky and Sayre, 1978; Bird et al., 2003).

Mankau (1975) compared reproduction of *Pr. scribneri* on *Phaseolus vulgaris* in greenhouse pot culture in soils with and without *Pa. penetrans*. Two soil treatments were prepared. Soil infested with 50 *Pr. scribneri* per 100 cm³ was mixed in a 1:1 (v/v) ratio with: (1) *Pa. penetrans* endospore-infested soil and (2) steam-sterilized greenhouse soil. Each soil treatment was divided among five 10-cm pots planted with green beans, which were then grown in a greenhouse. After 55 days, nematodes were recovered from roots and soil and counted with a dissecting microscope. The number of lesion nematodes recovered from endospore-infested soils was 244 ± 20.3 , compared to 607 ± 12.7 recovered from steam-sterilized soils, and differences were significant at $P = 0.01$.

Mankau and Prasad (1977) observed cross-generic parasitism of *Pa. penetrans* infecting *Meloidogyne*, *Pratylenchus brachyurus* and *Pr. scribneri*, but not *Pr. penetrans*. Elekçioğlu (1995) reported attachment of *Pasteuria* endospores to *Pratylenchus penetrans* but did not observe infection. Other reports of *Pasteuria* species on *Pr. penetrans* do not clarify whether or not infection was confirmed (Sayre et al., 1988). Chen and Dickson (1998) reported observations of *Pasteuria* attached to 12 lesion nematode species from around the world, two of which were *Pr. penetrans*. Gonzaga and Santos (2009) reported *Pasteuria* endospore attachment and infection of *Pr. brachyurus* and *Pr. zaeae*. Information on parasitism of *Pratylenchus penetrans* by *Pasteuria* is scarce and studies on interactions between these organisms are lacking.

Pasteuria Bioscience, Inc. in Florida successfully cultured *Pasteuria* using *in vitro* fermentation. This company was acquired by Syngenta Crop Protection, LLC in 2012. EconemTM was the first commercially available formulation of *Pasteuria usgae*. It was used as a

biological nematicide to treat the sting nematode, *Belonolaimus longicaudatus* in turf grass (Pasteuria Bioscience, Inc., 2009, but is no longer in commercial production. Commercial development of additional *Pasteuria*-based nematicides to control economically important plant-parasitic nematodes continues at Syngenta Crop Protection, LLC.

2.2 Biology of *Pratylenchus* Filipjev

The genus *Pratylenchus*, commonly known as root-lesion nematodes, has been reviewed by Loof (1978), Dropkin (1989), Handoo and Golden (1989), Zunke (1990), Mai et al. (1996), Barker et al. (1998), Shurtleff and Avere (2000), and Castillo and Volvas (2007). Lesion nematodes are 0.45 to 0.70 mm in length and have a sclerotized stylet that is 15 to 18 μ m in length. Key morphological features include a broad head with a flattened lip region, large metacarpus, and single ovary. The basal bulb of the esophagus overlaps the intestine lateroventrally.

Members of this genus parasitize a wide range of plants. Select species reduce yields of economically important agricultural and horticultural crops. Plant symptoms of parasitism include visible abrasions on roots, as well as root shedding, increased lateral roots, stunted plant growth, chlorosis, and plant death when parasitism is severe. Lesion nematodes feed on external and internal plant root tissues. After entering a plant host, nematodes migrate within and between cortical cells and consume cell contents, causing cell death and necrotic lesions on the root.

2.2.1 Life cycle

All life stages of lesion nematodes overwinter in roots or soil and, except for the egg and first-stage juvenile, are migratory and infective. These nematodes reproduce by parthenogenesis when males are absent or by amphimixis when males are present. Adult females deposit eggs in soil or within plant roots. The first juvenile larva, or J1, forms within the egg and molts into a

second stage juvenile (J2) which then hatches from the egg. The infective J2 migrates to a host root and feeds before molting into the third juvenile stage (J3) and then the fourth (J4). A final molt results in a reproductively mature adult. Under optimal conditions (suitable host plant, soil pH ranging from 5 to 8, and soil temperatures between 28°C and 30°C), the life cycle from egg to adult spans 25 days.

2.2.2 Ecology

Pratylenchus species are found in temperate, subtropical and tropical regions throughout the world. Population densities of lesion nematodes are affected by the biotic and abiotic factors of the soil ecosystem in which they are found. These soil parameters include microclimate (temperature and moisture), physical and chemical properties (soil type, percent organic matter, pH, cation exchange capacity, salinity, nutrient availability), and species richness. The distribution and population densities of plant-parasitic nematode species vary based on soil type, texture, moisture, and temperature, as well as host crop (Norton, 1978).

Pratylenchus is found in coarse, sandy soils and sandy loam soils. Field populations of some species often are reported above the damage threshold in many coastal regions. Kotcon (1990) found differences in lesion nematode densities associated with soil type. Species of lesion nematodes have been observed in soils with temperatures ranging from 5°C to 38°C (Shurtleff and Averre, 2000; Bhan et al., 2010).

Shukla et al. (1997) compared effects of three soil pH levels on reproduction of *Pratylenchus thornei* on spearmint (*Mentha spicata* L.). They reported lowest and highest final populations at pH 3 and 9, respectively. In a greenhouse pot trial, Willis (1972) compared reproduction of *Pr. penetrans* on alfalfa at four pH levels and observed greatest yield reduction and highest nematode reproduction at pH levels of 5.2 and 6.4.

Population densities and species richness of *Pratylenchus* varies with species and associated plant host. Based on observations of *Pr. penetrans* on 24 weed species, Bélair et al. (2007) reported highest lesion nematode reproduction on members of family Brassicaceae and lowest reproduction on members of family Cyperaceae. Kotcon (1990) reported seven species of *Pratylenchus* in soils sampled from peach orchards in the State of West Virginia.

Loof (1978) indicates several species of *Pratylenchus* often occur in mixed lesion nematode populations. Kotcon (1990) reported mixed populations of lesion nematodes and *Xiphinema* species in West Virginia peach orchard soils, and noted a positive correlation between population densities of *Hoplolaimus* and *Pratylenchus* species.

2.2.3 Distribution

At least 68 species of *Pratylenchus* parasitize more than 500 plant species and occur in and near tropical, subtropical, and temperate zones throughout the world. Mai et al. (1960) reported at least one species of *Pratylenchus* present in 12 states in the northeastern United States. Kotcon (1990) reported *Pratylenchus* species in 77% of samples surveyed from West Virginia peach orchards. Loof (1978) noted that the most common lesion nematode species, *Pr. penetrans*, *Pr. crenatus*, *Pr. vulnus*, *Pr. thornei*, *Pr. neglectus*, *Pr. brachyurus*, *Pr. zaeae*, *Pr. coffeae*, and *Pr. hexincisus* are widely distributed across all continents.

Pratylenchus penetrans and *Pr. crenatus* often occur in mixed populations and are reported among the five most common lesion nematode species in northeastern North America (Townshend et al., 1978). Mai et al. (1977) stated that *Pr. penetrans* “is the most economically important plant-pathogenic nematode in the northeastern states” of the US. It is also a significant pest of potato in several states of the Pacific Northwest, as well as Canada and Europe (McKeen and Mountain, 1960; Bélair et al., 2007; Smiley et al., 2008).

2.2.4 Management practices

Pratylenchus penetrans parasitizes more than 400 hosts, including conifer and fruit trees, strawberries, legumes, grasses, cereals, tomato, tobacco, potato, horticultural plants, and several weed species (Corbett, 1970; Corbett, 1973; Mai et al., 1977; Ferris and Ferris, 1998). These nematodes cause significant yield losses in many agricultural, horticultural, and forestry-related industries (Mai et al., 1977; Ferris and Ferris, 1998; Westerdahl et al., 2003; Bélair et al., 2007; Smiley et al., 2008). Some plant diseases caused by *Verticillium*, *Rhizoctonia*, and *Fusarium* show increased severity when *Pr. penetrans* is present, and this nematode is involved in other fungal, viral, and bacterial plant disease complexes (McKeen and Mountain, 1960; Loof, 1978; Dropkin, 1989; Jin et al., 1991; Barker et al., 1998; Saeed et al., 1998; Shurtleff and Avere, 2000; LaMondia, 2003).

In a survey of density and frequency of plant-parasitic nematodes recovered from crop production soils, Anwar et al. (2010) reported *Pr. penetrans* in 30% of 325 soils sampled from 19 vegetable crops. They found that population densities of *Pr. penetrans* and *M. incognita* were at “potentially damaging levels in most of the vegetable crops surveyed.” Hollaway et al. (2008) detected *Pr. penetrans* in Australian cereal crop soils and were concerned about the lack of current management options based on prior reports of damaging *Pr. penetrans* infestations in pea, wheat, and oat.

Pest management practices used to control most plant-parasitic nematodes are ineffective for controlling *Pr. penetrans* (Mai et al., 1977; Cook and Baker, 1983; Bélair et al., 2007). Lesion nematodes may survive more than a year in the absence of a host (Sher and Allen, 1953; Loof, 1961; Dropkin, 1989; Bélair et al., 2002). Cobb (1917) notes that their broad host range can compromise efficacy of crop rotation in infested field soils. Chemical nematode controls are

available for some, but not all, conventional cropping systems, and demand for biological controls to manage these pests is increasing.

A few studies verify effective biological control of *Pr. penetrans*. Some success has been observed with cropping sequences that rotate specific varieties of plants which are either poor hosts or are known to be suppressive to lesion nematodes (Bélair et al., 2007; Bhan et al., 2010). Examples of plants that reduce populations of *Pr. penetrans* include species of marigold (*Tagetes patula* and *T. erecta*), hybrid cultivars of forage and grain pearl millet (*Pennisetum glaucum* L.), wheat (*Triticum aestivum* cv. Stacy), and oat (*Avena strigosa* cv. Saia) (Loof, 1978; Dropkin, 1989; Ball-Coelho et al., 2003; Bélair et al., 2005; LaMondia, 2006). In Canada, cropping systems utilize pearl millet as biocontrol of lesion nematodes on potato (Bélair et al., 2005).

Additional biological control agents and crop production practices are being investigated as alternatives to chemical treatments that reduce populations of *Pr. penetrans* (Westerdahl et al., 2003; Pal and McSpadden Gardener, 2006; Bélair et al., 2007). Hackenberg et al. (2000) reported suppression of *Pr. penetrans* on apple seedlings as well as in field and pot cultures of strawberry treated with *Pseudomonas chlororaphis* strain Sm3. Timper and Brodie (1994) indicated inconsistent suppression of *Pr. penetrans* by *Hirsutella rhossiliensis*. However, additional studies of interactions between this fungal species and lesion nematodes are warranted.

Endospores of *Pasteuria* have been reported on lesion nematodes for several decades. Infection of *Pr. brachyurus* by *Pasteuria thornei* is well documented. The current and potential threat that *Pratylenchus penetrans* poses to agricultural crop production warrants further investigations of the interactions between this highly virulent species and *Pasteuria* species.

2.3 Organic Agriculture

United States Federal Code defines organic production as, “A production system that is managed in accordance with the Act and regulations in this part to respond to site-specific conditions by integrating cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity” (USDA, 2010). The term “organic farming” was first used in 1941, when Northbourne described it as the management of all aspects of a farming system incorporating animals, plants, civilization, and the soil (Lotter, 2003). Conventional agriculture systems rely heavily on synthetic fertilizers and pesticides, such as nematicides, to maintain and increase crop production, whereas organic systems do not (Lotter, 2003; Pimentel et al., 2005).

Research comparing organic and conventional farming systems indicates reduced environmental and human health risks associated with organic practices compared with conventional methods (Reganold et al., 2001; Lotter, 2003; Pimentel, 2005; Pimentel et al., 2005). Based on a 22-year study of organic and conventional cropping systems, Pimentel et al. (2005) reported that organic cropping systems reduce chemical pollutants, fossil energy inputs, soil erosion, and surface runoff and increase soil organic matter, soil biodiversity, water conservation, and drought tolerance.

Consumers are increasingly aware of the ecological and nutritional benefits of sustainably manufactured products, and the demand for organic goods continues to rise (Lotter, 2003; Sooby et al., 2007; International Federation of Organic Agriculture Movements (IFOAM), 2012). In 2011, worldwide sales of organic goods exceeded \$62 billion, a four-fold increase since 1999 (IFOAM, 2012). Nearly half of these sales were in the United States, which is the leading consumer of organically manufactured products (IFOAM, 2012). The fruit and vegetable

market is the highest area of consumer demand for organic products, accounting for 43% of demand in 2012 (USDA/ERS, 2015). To meet growing consumer demand, organic growers must increase yields while using natural, non-chemical methods mandated by government guidelines for certified organic crop production (Lotter, 2003; Sooby et al., 2007; USDA, 2010).

Annual global economic losses associated with damage and diseases caused by plant-parasitic nematodes have been estimated at 12 billion - 100 billion dollars (Sasser and Freckman, 1987; Bird and Koltai, 2000). Damage caused by plant disease depends on the damage threshold, which is the pest population level that the crop can withstand before yield losses occur. Some chemical pesticides used to control plant-parasitic nematodes require higher application dosages than the amount needed to manage other agricultural pests (Johnson and Feldmesser, 1987; Thomason, 1987). Evidence of environmental and human health risks associated with these treatments has resulted in restricted or revoked use of most commercial nematicides (Johnson and Feldmesser, 1987; Thomason, 1987; Nordmeyer, 1992; Pimentel, 2005).

The United States Environmental Protection Agency (EPA) banned use of nematicidal soil fumigants containing 1,2-dichloropropane (1,2-D), ethylene dibromide (EDB), and 1,2-dibromo-3-chloropropane (DBCP) in the 1980s. Since then, the majority of pesticides used for nematode control have been discontinued by the manufacturer or restricted to non-nematicidal use (Johnson and Feldmesser, 1987; Nordmeyer, 1992). Aldoxycarb, fenamiphos, and carbofuran were cancelled or discontinued in 2005, 2007, and 2009, respectively. The remaining nematicides, such as methyl bromide, metam-sodium, and 1,3-dichloropropene (1,3-D), also are at risk for cancellation or restricted use in the future (Thomason, 1987; Nordmeyer, 1992).

Organic agriculture replaces use of conventional chemical pesticides with use of biological control practices that promote plant vigor and reduce populations of plant pests and

pathogens. Innovative pest management methods and biological alternatives to chemical nematicides are needed to control plant-parasitic nematodes in both organic and conventional farming systems (Pimentel, 2005). Investigations of the biological control potential of naturally occurring antagonists of plant pathogens, which have been identified from field soils, are underway throughout the world. Continued studies of natural plant-disease suppression, especially in organic crop production soils, may lead to discoveries of additional biological controls for plant-parasitic nematodes (Kerr, 1982; Cook and Baker, 1983; Jatala, 1986; Sikora, 1992; Stirling, 1992; Hallman et al., 2007).

The West Virginia University Horticulture Research Farm was established in 1916 for conventional crop production research. The farm was designated for organic agriculture research in 1999, after which a transition from conventional to organic production began. The WVU Organic Research Farm has been certified organic by the Ohio Ecological Food and Farm Association since 2003. Although specific nematode management has not been implemented, populations of plant-parasitic nematodes in these soils have not increased in the presence of suitable host crops (Kotcon, 2006; Kotcon, 2010). This suggests these soils are suppressive to plant-parasitic nematodes, including *Pratylenchus*. Although endospores of *Pasteuria* were observed previously on root-lesion nematodes from these soils, the nature of suppression was not determined.

2.4 Biological Control and Disease Suppressive Soils

The objective of biological control is to utilize a natural, non-chemical approach to reduce severity of plant diseases and increase crop yield (Kerr, 1982; Jatala, 1986; Kerry, 1990; Sikora, 1992; Stirling, 1992; Pal and McSpadden Gardener, 2006; Stevens and Stuart, 2008). Biological control in agricultural crop production has been loosely defined as any practice that

preserves, increases, or introduces naturally occurring antagonists of plant pathogens. A biological control agent is a living organism that disrupts infection or infestation of a plant host by one or more pathogens (Norton, 1978; Kerr, 1982; Cook and Baker, 1983; Pal and McSpadden Gardener, 2006).

Sikora (1992) described two types of biological control practices. Augmentative biocontrol practices are “the ‘classical’ approach of managing the antagonistic potential ... of a soil” and inundative biocontrol systems are “based on repeated application of biological agents” (Sikora, 1992). Therefore, augmentative practices may increase existing populations of *Pasteuria* present in soils; whereas, inundative methods involve direct application of *Pasteuria* endospores into soils. Augmentative biocontrol methods involve the use of soil amendments to suppress pathogens and enhance plant growth. These practices increase soil quality, organic matter, and populations of native organisms to maintain or promote natural disease suppression in cultivated soil systems (Sikora, 1992; Lotter, 2003; Janvier et al., 2007). Crops cultivated in soils amended with organic matter grow vigorously and maintain natural host defenses against plant pathogens (Coyne and Thompson, 2006; Pimentel et al., 2005; Janvier et al., 2007). Amended soils have higher populations of indigenous soil organisms, such as beneficial microorganisms and antagonists of plant pathogens, compared to non-amended soils (Sikora, 1992; Pimentel et al., 2005; Coyne and Thompson, 2006; Janvier et al., 2007).

Based on greenhouse and field studies of four types of organic amendments tested in cocoa production soils, Fademi et al. (2010) reported induced suppression of root-knot nematodes in all amended soils, but no suppression in non-amended control treatments. In addition to organic soil amendments, examples of augmentative biocontrols include mechanical weed control, monitoring pest populations, choosing disease-free seed and rootstock,

intercropping and rotating crops, and planting disease resistant cultivars (Mankau, 1968; Sikora, 1992; Coyne and Thompson, 2006; Janvier et al., 2007).

Inundative methods involve direct application of beneficial organisms and/or biological control agents to reduce pathogen populations. Biocontrol agents are a specific class of beneficial organisms that reduce plant disease by direct antagonism of pests. Other types of beneficial organisms, such as some rhizobacteria species, promote plant vigor and support natural plant host defense mechanisms.

Biocontrol agents include select viruses and species of bacteria, fungi, and invertebrates that are naturally antagonistic to plant pathogens (Norton, 1978; Kerr, 1982; Cook and Baker, 1983; Stirling, 1992; Pal and McSpadden Gardener, 2006). Pioneer uses of commercial biocontrol agents occurred in 1962, with the use of *Peniophora gigantea* to control *Heterobasidium annosum*, and in 1973, with the use of *Agrobacterium radiobacter* strain 84 to control *Agrobacterium tumefaciens* (Kerr, 1982). Some known biocontrol agents include *Bacillus*, *Pasteuria*, *Pseudomonas*, and *Streptomyces* species, as well as fungi such as *Aspergillus flavus*, *Coniothyrium minitans*, *Venturia inequalis*, *Trichoderma*, and *Gliocladium* species (Kerr, 1982; Cook and Baker, 1983; Dong and Zhang, 2006; Pal and McSpadden Gardener, 2006; Tian et al., 2007).

While many of these microbes have been formulated into biological pesticides that target bacterial and fungal plant pathogens, only a few target plant-parasitic nematodes. Biological nematicides have been formulated and are registered with the US EPA and approved by the Organic Materials Review Institute for use in organic agriculture. Examples include DiTera, a product of fermented *Myrothecium verrucaria*, MeloCon WG, a formulation of *Paecilomyces lilacinus* strain 251, and Nema-Q, an extract of *Quillaja saponaria*.

Few studies have investigated biological control of plant-parasitic nematodes in organic crop production soils. Pokharel and Zimmerman (2010) compared nematode populations recovered from two peach cultivars planted in organic and conventional peach orchards. Average population densities of plant-parasitic nematodes in soils from both peach varieties were lower in the conventional orchard than in the organic orchard. Authors noted the highest densities of plant-parasitic nematodes occurred in soils from one of the conventionally grown varieties. Diversity of bacterial and fungal feeders, as well as plant-parasitic nematodes, was greater in soils from both cultivars in the organic orchard than in the conventional orchard. These authors detected species in five genera of plant-parasitic nematodes including *Pratylenchus* and *Meloidogyne*.

Atkins et al. (2003) demonstrated that the nematophagous fungus, *Pochonia chlamydosporia* var *catenulate*, reduced populations of root-knot nematodes in organically managed soils when combined with crop rotation. Combinations of Neem oil and organic amendments, as well as treatments using *Paecilomyces lilacinus*, show promise for use in biological control of *Meloidogyne* species (Rao et al., 1997; Oka et al., 2007). Neem oil is extracted from the leaves of the Neem tree, *Azadirachta indica*, and may have potential for biocontrol of plant-parasitic nematodes.

2.4.1 Suppressive soils

Discovery of new biological control agents often begins with observation of prolonged periods of low disease incidence in the presence of suitable host crops, especially in untreated soils such as those found in organic cropping systems (Cook and Baker, 1983; Sikora, 1992; Lotter, 2003). Disease suppressive soils maintain plant pathogen populations below the damage threshold in the presence of susceptible hosts, and these soils have been identified in organic,

conventional, and non-cultivated systems (Shipton et al., 1973; Cook and Baker, 1983; Barker et al., 1998; Westphal and Becker, 2000; Pyrowolakis et al., 2002; Rimé et al., 2003; Bao et al., 2010; Kotcon unpub.). Conducive soils, in contrast, allow plant pathogen populations to increase above the damage threshold and cause significant yield losses.

Sikora (1992) stated that “the level of antagonism in soil may vary from total suppression to stimulation of a specific nematode pest or disease.” In some cases, controlled experiments reveal biotic causes of suppression and lead to isolation of one or more potential biocontrol agents, which may then be evaluated for suppressive or antagonistic effects upon targeted plant pathogens (Cook and Baker, 1983; Sayre, 1986; Stirling, 1992; Barker et al., 1998; Pal and McSpadden Gardener, 2006). General suppression is attributed to a range of living and non-living soil factors; whereas, specific soil suppressiveness is caused by one living organism (Cook and Baker, 1983; Pal and McSpadden Gardener, 2006). Soils with specific nematode suppressiveness have been identified at several field sites (Dickson et al., 1992; Kluepfel et al., 1993; Westphal and Becker, 2000; Pyrowolakis et al., 2002; Westphal, 2005).

A determining factor for specific suppression is transferability of disease suppressiveness to non-suppressive or disease conducive soils (Cook and Baker, 1983). Shipton et al. (1973) reported transfer of microbial antagonism of take-all decline in wheat by adding 1% antagonistic soil to fumigated field and steamed greenhouse soils. Sterilized soils inoculated with *Pasteuria penetrans*-infested soil can develop suppressiveness to *Meloidogyne incognita* in greenhouse pot culture (Mankau, 1975). A number of subsequent reports indicated that specific soil suppression of root-knot nematodes by *Pasteuria* species was likely based on transferability of suppression (Oostendorp et al., 1991a; Chen et al., 1996b; Weibelzahl-Fulton et al., 1996; Freitas et al., 2000; Kariuki and Dickson, 2007).

2.5 Conclusion

A variety of biological control products are available to reduce bacterial and fungal plant pathogens; however, development of biological nematicides and alternative methods for suppression of plant-parasitic nematodes is needed. Recent research indicates that species of *Pasteuria* have the potential to meet this need for nematode biocontrol. Interactions between *Pasteuria* and select species of economically important plant-parasitic nematodes have been studied, yet only a minor fraction of this research has been conducted on *Pratylenchus* species.

3.0 RESEARCH OBJECTIVES

Objective 1. Assess the geographic distribution and frequency of *Pasteuria* and *Pratylenchus* species in certified organic crop production soils collected from the Mid-Atlantic region of the United States. Population density of *Pratylenchus* will be correlated with the following soil variables: presence of *Pasteuria*, soil type, soil pH, cation exchange capacity, and mineral availability of potassium, phosphorus pentoxide, calcium, and magnesium.

Objective 2. Determine if nematode suppressiveness of West Virginia University Organic Research Farm soils is caused by biotic or abiotic factors and determine effects of soil volume on density of *Pratylenchus penetrans* in pot culture. Mean density of *Pratylenchus penetrans* infecting *Phaseolus vulgaris* cv. Jade is expected to be lower in non-sterile than in sterile soil treatments, but not statistically different among soil volume treatments.

Objective 3. Test the hypothesis that the mean rate of increase of *Pratylenchus penetrans* populations infecting *Phaseolus vulgaris* cv. Jade will be lower in soils containing *Pasteuria*-attached *Pratylenchus* nematodes than in soils where *Pasteuria* was not detected ($P = 0.10$).

4.0 MATERIALS AND METHODS

To test Objective 1, soils from organic farms in the Mid-Atlantic region were collected to determine presence of *Pa. penetrans*. A greenhouse baiting procedure was conducted on a subset of these samples. Lab bench trials were conducted to assess Objectives 2 and 3. Survey procedures are described in section 4.1, below. Nematode culture procedures are described in section 4.2. Experimental design, plant growth conditions, harvest methods, and data analysis procedures are described in sections 4.3, 4.4, 4.5, and 4.6, respectively.

Note: Nematode specimens of interest in these studies were photographed on 35-mm film using a Nikon OPTIPHOT differential interference contrast microscope fitted with a Nikon AFX-II camera. However, these images are not included because the 35-mm film was double-exposed and all photographs were discarded.

4.1 Collection of soils

The survey of *Pasteuria* and *Pratylenchus* in organic cropping soils was conducted based on methods modified from Kotcon (1990). Soils were collected from crop-growing areas at certified organic farms in Maryland, Ohio, Pennsylvania, Virginia, and West Virginia (Table 4.1).

Between March and June 2008, certified organic growers were visited and interviewed to collect information regarding crop history and pest management practices. One to three 19-liter soil samples were collected from each farm. Each 19-liter sample was a composite of multiple samples taken from varying locations in the same crop growing area. A spade-tipped shovel was used to collect a 3- to 4-liter soil sample to a depth of 15 to 20 cm below the soil surface. Each sample was assigned an identification code indicating the farm where the sample was collected

and the number of samples collected at that farm. Soils were transported to the WVU Nematology laboratory and stored at $11 \pm 3^{\circ}\text{C}$ for 2 to 4 weeks.

In November 2008, a 19-liter soil sample was collected from WVU Organic Research Farm vegetable plots using the same method described herein. The soil sample was stored in the WVU Nematology laboratory at $20 \pm 3^{\circ}\text{C}$ for 6 days.

Visible debris and macro organisms were removed and each soil sample was hand mixed for 5 to 6 minutes. A 500-cm^3 subsample was stored at $11 \pm 3^{\circ}\text{C}$ for nematode extraction and a 20-cm^3 subsample was analyzed by the WVU Soil Testing Laboratory to determine soil pH, cation exchange capacity, and cation concentrations. Remaining soil was stored at $11 \pm 3^{\circ}\text{C}$ for use in future studies. Soil types and textures were determined based on the geographic location of each farm using the USDA Natural Resources Conservation Service Web Soil Survey (2009).

Table 4.1. Fifteen counties from five Mid-Atlantic states represented in the survey of *Pasteuria* on *Pratylenchus* from certified organic cropping soils.

State	County or Counties
Maryland	Baltimore, Frederick, Washington
Ohio	Athens, Noble, Wayne
Pennsylvania	Allegheny, Beaver, Butler, Huntingdon, Indiana, Somerset, Westmoreland
Virginia	Madison
West Virginia	Preston

4.2 Host nematode cultures

Inocula consisting of mixed life stages of *Pratylenchus penetrans* were obtained from two sources. A population of *Pr. penetrans* established from a single female isolated from *Prunus* sp. was maintained on *Zea mays* cv. Doeblers N659 in WVU greenhouse pot cultures. This population was maintained by harvesting corn plants at 8-week intervals and direct-seeding corn into nematode infested soil. Nematodes extracted from this culture were used for the preliminary experiment on WVU Organic Research Farm soils. Nematodes were extracted from

corn roots using the shaker extraction method for 9 days followed by incubation of roots on Baermann funnels for an additional 10 days (Niblack and Hussey, 1985; Barker et al., 1986).

Axenic root explant cultures of *Pr. penetrans* on *Zea mays* (IO Chief) were provided by the USDA Agricultural Research Service Nematology laboratory in Beltsville, MD. This population was established from a single *Pr. penetrans* female isolated from lawn soils at USDA-ARS, Beltsville, MD (L. Carta, pers. commun.). This population of *Pr. penetrans* (ARS-MD isolate) was increased by aseptically transferring subsections of nematode-infested root explants from established plates to fresh corn root explants on potato dextrose agar. Culture plates were stored in the WVU Nematology laboratory at $20 \pm 3^{\circ}\text{C}$. This population was used for the experiment that compared reproduction of *Pr. penetrans* in soils with and without *Pasteuria*. Nematodes were extracted from corn root explants in agar using the Baermann funnel method (Niblack and Hussey, 1985; Barker et al., 1986).

4.3 Experimental design and preparation of treatments

4.3.1 Increasing detection of *Pasteuria*

Some of the soils collected for the survey of lesion nematodes and *Pasteuria* were suspected to have *Pasteuria* at levels below detection because endospores were observed on *Pratylenchus* in samples taken from other cropping areas at the same farm. A greenhouse baiting trial was conducted to increase detection of *Pasteuria* on *Pratylenchus* in six soil samples. For each soil sample, 600 cm³ of untreated soil was hand mixed and then divided to fill two 400-cm³ plastic pots with 300 cm³ soil in each. Corn (*Zea mays*) cv. Doebblers N659 and green bean (*Phaseolus vulgaris*) cv. Jade were each direct-seeded into separate pots of the same soil.

4.3.2 Reproduction of *Pr. penetrans* in four soil treatments

To assess differences in reproduction of *Pratylenchus penetrans* in sterilized versus non-sterilized WVU Organic Research Farm soil, methods described by Timper and Brodie (1994), Weibelzahl-Fulton et al. (1996), Hackenberg et al. (2000), Bélair et al. (2007), and Jaffee et al. (2007) were modified. Four soil treatments were prepared with soil collected from the WVU Organic Research Farm: (i) sterilized soil, (ii) non-sterile soil, (iii) 90:10 soil (90% sterile and 10% non-sterile soil), and (iv) a non-sterile, uninoculated soil.

Two types of planting containers were used: (i) 400 cm³ of soil treatments in 500-cm³ plastic pots, or (ii) 150 cm³ of soil treatments in 250-cm³ glass beakers.

Sterile sand was mixed 1:1 (v/v) with the soil sample and moistened. The sterile soil treatment was prepared by steaming a 4,000-cm³ subsample in an autoclave at 80°C for 70 minutes (Chen et al., 1996b). The remaining untreated soil was stored in the laboratory at 20 ± 3°C. After autoclaving, the sterilized soil cooled on the laboratory bench for 24 ± 3 hours. Soil treatments were then added to the planting containers. Three replicates each of the four soil treatments in the two types of planting containers were arranged in a randomized factorial experiment conducted on a bench top in the WVU Nematology laboratory. Green beans were germinated in sterile soil at 20 ± 3°C for 11 days, with one seedling planted per container.

The first three treatments were inoculated with a suspension of 58 ± 6 *Pr. penetrans* inoculum prepared as described in section 4.2, and the fourth treatment served as an uninoculated control. The uninoculated control treatments were added to the bench 4 weeks after the three inoculated treatments were planted.

4.3.3 Reproduction of *Pr. penetrans* in soils with and without *Pasteuria*

Six soils containing *Pasteuria* endospores were matched with soils of similar soil pH, soil type and soil texture in which *Pasteuria* endospores were not detected (Table 4.2). A total of 144

pots were arranged in three blocks of eight soil treatments in a randomized complete block, four-way factorial design. The blocking variable was time between planting, and treatment variables were 1) presence or absence of *Pasteuria*, 2) soil with or without inoculum consisting of 277 ± 11 mixed life stage nematodes of *Pr. penetrans*, 3) harvest time of 2 or 8 weeks, and 4) soil type.

Sterile sand was mixed 1:1 (v/v) with each soil sample. A 9-cm³ subsample was analyzed to determine percent organic matter content (carbon and nitrogen) in the WVU Soil Chemistry Laboratory under the direction of Dr. Louis M. McDonald (Table 4.2). Green beans cv. Jade were germinated in sterile sand for 5 days at $20 \pm 3^\circ\text{C}$. Sterilized plastic pots were filled with 1,000-cm³ of the sand/soil mixture. Soils were dried in pots for 19 ± 3 hours at $66 \pm 16^\circ\text{C}$ in drying ovens to reduce background populations of lesion nematodes without affecting viability of *Pasteuria* endospores (Timper and Brodie, 1994; Jaffee et al., 2007). Each pot contained one green bean seedling.

Table 4.2. Soil pH, soil type, soil texture and organic matter content (percent carbon and nitrogen) of six soil pairs used in an experiment to compare reproduction of *Pratylenchus penetrans* on *Phaseolus vulgaris* in soils with and without *Pasteuria*.

Sample Code	Soil Pair	<i>Pasteuria</i> detected (+/-)	Soil pH	Soil Type	Soil Texture	% Carbon (OM)	% Nitrogen (OM)
MD2A	1	+	6.4	Hapludalf	Fine	2.29	0.18
MD24	1	-	6.3	Hapludalf	Fine-loamy	1.30	0.06
MD2B	2	+	7.2	Hapludalf	Fine	2.11	0.13
MD4	2	-	7.2	Hapludalf	Fine	3.57	0.33
PA17A	3	+	6.4	Hapludult	Fine-loamy	2.11	0.12
PA18	3	-	6.7	Hapludult	Fine-loamy	3.09	0.21
PA15C	4	+	6.9	Fragiudalf	Fine-loamy	2.66	0.20
MD22	4	-	6.4	Fragiaqualf	Fine-silty	1.26	0.06
PA11B	5	+	6.5	Dystrochrept	Loamy-skeletal	1.64	0.10
PA12	5	-	6.9	Endoaquult	Fine	2.22	0.23
PA16	6	+	5.5	Dystrochrept	Loamy-skeletal	1.58	0.11
PA17C	6	-	6.0	Fragiudult	Fine-loamy	1.61	0.15

Soil types and textures were determined using the USDA NRCS Web Soil Survey (<http://websoilsurvey.nrcs.usda.gov>). Organic matter analyses were performed by the WVU Soil Chemistry Research Laboratory under the direction of Dr. Louis M. McDonald.

4.4 Plant growth conditions

4.4.1 Increasing detection of *Pasteuria*

Green bean and corn plants were thinned to one plant per pot after 7 days and were grown in the greenhouse at $23 \pm 2^{\circ}\text{C}$. Green bean and corn were harvested after 9 and 12 weeks, respectively.

4.4.2 Reproduction of *Pr. penetrans* in four soil treatments

One 400-watt metal halide lamp (Sunlight Supply, Inc. Vancouver, WA, USA) placed 112 cm above soil surfaces provided supplemental light with an 8-hour photoperiod. Light intensity was not measured. Soil moisture content was determined by comparing the weight of a 10-cm^3 subsample before and after drying in an oven at 45°C for 24 hours, and plants were watered daily to maintain soil moisture at approximate field capacity.

4.4.3 Reproduction of *Pr. penetrans* in soils with and without *Pasteuria*

Four metal halide lamps (P.L. Light Systems, Beamsville, ON, Canada LOR 1B1) placed 112 cm above soil surfaces provided supplemental light with a 10-hour photoperiod (Kotcon et al., 1987). Light intensity was not measured. The average amount of water lost from all plants in a 24-hour period was calculated every 3 weeks and used to determine the volume of sterile distilled water to add to each plant per day.

4.5 Harvest methods and nematode assays

4.5.1 Survey of *Pasteuria* on *Pratylenchus* in certified organic cropping soils

Nematodes were extracted from soil using the modified sucrose-centrifugation method, and extraction solutions were stored at 11°C for observation (Jenkins, 1964; Panaccione et al., 2006). Nematodes were examined at 60 to 120 X magnification with a Nikon SMZ660 stereo light microscope. Nematodes of interest were hand-picked and prepared for oil immersion

microscopy, which was performed with a Nikon OPTIPHOT differential interference contrast light microscope at 125 to 600 X magnification. Nematodes examined under oil immersion were observed for attachment of *Pasteuria* endospores and identified to genus and species. Population density and frequency of *Pratylenchus* and *Pasteuria* were recorded. The number of lesion nematodes was extrapolated from partial counts for two samples (PA14 and PA16).

4.5.2 Increasing detection of *Pasteuria*

To extract nematodes from roots, plants were destructively harvested and roots were rinsed with tap water and blotted dry. Fresh root weight was recorded. Up to 1 gram of root was placed in a 125-ml flask with 50 ml of tap water, and flasks were incubated on a rotary shaker at 150 rpm at $20 \pm 3^\circ\text{C}$ for 4 days (Barker et al., 1986). Each shaker solution was poured through a 710- μm -pore sieve to remove root debris. Nematodes were collected on a 15- μm -pore sieve, transferred to a test tube, and stored at $11 \pm 3^\circ\text{C}$ for 1 to 6 days (Barker et al., 1986).

Lesion nematodes extracted from roots were counted, microscopically examined for attachment of *Pasteuria* endospores, and identified to species, when possible, using the same methods previously described. Nematodes were not recovered from soils.

4.5.3 Reproduction of *Pr. penetrans* in four soil treatments

Plants were harvested 8 weeks after transplanting. Up to 1 gram of root was incubated on a shaker, and nematodes were extracted at 3-day intervals for 9 days. Nematodes were examined at 60 to 120 X magnification with a Nikon SMZ660 stereo light microscope. One to 14 lesion nematodes per sample were examined for *Pasteuria* endospores. Population density and frequency of *Pratylenchus* and *Pasteuria* were recorded. When less than 1 gram root weight was recovered from a harvested plant, densities of *Pratylenchus* species, *Pr. penetrans*, and *Pr. crenatus* per gram root were extrapolated from actual counts.

4.5.4 Reproduction of *Pr. penetrans* in soils with and without *Pasteuria*

Half of the 48 plants in each block were harvested at 2 weeks after planting, with the remaining plants harvested at 8 weeks after planting. Fresh weight of the roots of each plant was measured. The shaker method (Kotcon et al., 1987) was used to extract nematodes from up to 1 gram root. Nematodes were recovered from shaker solutions at 2-day intervals for 4 days. For the 8-week harvest, fresh shoot weights were recorded, then oven-dried at 40°C for 5 days to determine shoot dry weight.

Soils were stored in zip closure bags at $11 \pm 3^{\circ}\text{C}$ for 7 to 10 days, and nematodes in soil were extracted using the modified sucrose-centrifugation method (Jenkins, 1964; Panaccione et al., 2006). Nematodes from root and soil samples were examined with a Nikon SMZ660 stereo light microscope at 60 to 120 X magnification, and all lesion nematodes were prepared for oil immersion microscopy with a Nikon OPTIPHOT differential interference contrast light microscope at 600 X magnification. Nematodes were identified to species and scanned for presence of *Pasteuria* endospores.

4.6 Data analyses

For the survey of *Pasteuria* on lesion nematodes in certified organic cropping soils, data were divided into three subsets based on presence of *Pratylenchus* and presence or absence of *Pasteuria* on lesion nematodes. Means, standard error, and correlation coefficients for soil parameters were calculated. R-values were significant at or above |0.33|. The assay conducted to increase detection of *Pasteuria* on lesion nematodes was not replicated and data analyses were not performed. Results are based on observations of nematodes found in roots from one plant grown in a single pot per each subsample of soil. The number of lesion nematodes recovered for samples MD2A and PA17A were not included for analyses of *Pratylenchus* densities in the

survey data. Detection of *Pasteuria* on *Pratylenchus* in sample MD2A was included in the survey data.

For the experiment that compared reproduction of *Pr. penetrans* in four soil treatments, one-way and two-way interactions were analyzed using the Least Squares Model with statistical significance at $P \leq 0.05$.

For the experiment that compared reproduction of *Pr. penetrans* in soils with and without *Pasteuria*, densities of *Pr. penetrans* in the 2- and 8-week harvests were compared to determine treatment effects on lesion nematode populations. Total nematode density per plant was calculated as a sum of the products of the number of nematodes extracted per unit of extraction (≤ 1 gram root or 100-cm³ soil), total fresh root weight, and total soil volume (Kotcon et al., 1987). Data were log transformed ($\log_{10}[x + 1]$) to normalize zero counts, and treatment effects and interactions were analyzed using four-way analysis of variance. One-way, two-way, three-way and four-way interactions were analyzed using the Least Squares Model and were considered statistically significant at $P \leq 0.10$. Unless otherwise noted, data were reported as untransformed means.

All data analyses were performed using JMP statistical software (SAS Institute Inc., Cary, North Carolina, USA).

5.0 RESULTS

5.1 Survey of *Pasteuria* on *Pratylenchus* in certified organic cropping soils

Pratylenchus were present in 22 of 34 samples from 25 farms in Maryland, Ohio, Pennsylvania, Virginia, and West Virginia (Figure 5.1). The geographic locations of the farms included in the survey was dependent upon grower response to solicitation.

Species were not determined for two samples due to absence of adult nematode stages. The number of lesion nematodes recovered ranged from one to 80 in the 22 samples in which they were present (Figure 5.2). For all but two samples, all lesion nematodes recovered were observed for endospores of *Pasteuria*. For sample PA14, 20 *Pratylenchus* were observed out of an estimated 80 total lesion nematodes recovered. For sample PA16, 10 of an estimated 20 lesion nematodes were observed. Nematode specimens lost or damaged during processing were reported as “*Pratylenchus* species unknown.”

Endospores of *Pasteuria* were observed on lesion nematodes recovered from field populations in five soil assays and from roots in two samples, one of which was identified in the baiting trial to increase detection of *Pasteuria* (Table 5.1). The proportion of *Pratylenchus* with *Pasteuria* endospores ranged from 20% to 75% for soil extractions and 10% to 50% for root extractions (Table 5.2).

Mean population density of *Pratylenchus* nematodes among all 22 samples was 9.2 ± 3.6 per 100 cm³ soil. Densities were higher in the 16 soils without *Pasteuria* than in soils where *Pasteuria* was detected on lesion nematodes (Table 5.3); however, differences between means were not significant (Student's t-test, $P = 0.68$).

Correlations between lesion nematode density and tested soil parameters were not significant when all 34 samples were included in the analysis. Population density of *Pratylenchus* was positively correlated with soil pH, cation exchange capacity and calcium when only the 22 samples with lesion nematodes were analyzed (data not shown). In the 16 soils in which *Pasteuria* were not detected on *Pratylenchus*, density of lesion nematodes was positively correlated with soil pH, cation exchange capacity, calcium, and phosphorus content (Table 5.4). Lesion nematode population density in six soils with *Pasteuria* was negatively correlated with

soil pH, calcium, and phosphorus content, and positively correlated with magnesium content (Table 5.5). The range of soil pH levels for all samples was 4.9 to 7.3 and the mean was 6.5 (data not shown).

Organic growers emphasize high diversity of rotation and cover crops, so no statistical analysis of host crop history was possible. Host crop history is listed in Appendix A.

One lesion nematode from sample MD2B was suspected to be *Pr. neglectus*, but key morphological characteristics were not confirmed due to poor specimen quality. Additional nematode genera observed from these soils included *Criconemoides*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Mononchus*, *Paratylenchus* and *Tylenchus*. In sample PA15C, endospores of *Pasteuria* were detected on both *Pratylenchus* and *Hoplolaimus*. Endospores of *Pasteuria* were not observed on lesion nematodes but were observed on *Helicotylenchus* in sample OH8 and on *Hoplolaimus* in sample PA19.

Figure 5.1. Distribution of *Pratylenchus* and *Pasteuria* on *Pratylenchus* in soils collected from certified organic farms in Maryland, Ohio, Pennsylvania, Virginia, and West Virginia.

Figure 5.2. Frequency of *Pratylenchus* nematodes recovered from 100-cm³ soil for 34 samples collected from certified organic farms in the Mid-Atlantic states.

Table 5.1. Frequency of *Pasteuria* endospores attached to *Pratylenchus* recovered from 100-cm³ soil or from 1-gram *Zea mays* roots or *Phaseolus vulgaris* roots for six samples collected from certified organic farms in the Mid-Atlantic states.

Sample ID	Specimen No.	No. of <i>Pasteuria</i> endospores observed	Species of nematode specimen (if known)	Source of specimen
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Survey of *Pasteuria* on *Pratylenchus* in organic farm soils:

MD2B	1	10	<i>Pr. crenatus</i>	Soil
PA11B	1	1	<i>Pr. crenatus</i>	Soil
	2	1	<i>Pr. crenatus</i>	Soil
	3	1	<i>Pr. crenatus</i>	Soil
PA15C	1	2	<i>Pr. crenatus</i>	Soil
PA16	1	1	<i>Pr. crenatus</i>	Soil
	2	2	<i>Pr. crenatus</i>	Soil
PA17A	1	1	<i>Pr. crenatus</i>	Soil

Baiting trial to increase detection of *Pasteuria*:

PA17A	2	1	<i>Pratylenchus</i> sp.	Corn roots
MD2A	1	1	<i>Pr. crenatus</i>	Corn roots
	2	1	<i>Pr. crenatus</i>	Corn roots
	3	2	<i>Pr. crenatus</i>	Corn roots
	4	2	<i>Pratylenchus</i> juvenile	Corn roots
	5	1	<i>Pratylenchus</i> juvenile	Bean roots
	6	1	<i>Pratylenchus</i> juvenile	Bean roots

Table 5.2. Proportion of *Pratylenchus* with and without *Pasteuria* recovered from 100-cm³ soil or from 1-gram *Zea mays* roots or *Phaseolus vulgaris* roots for six samples collected from certified organic farms in the Mid-Atlantic states.

Sample ID	No. of lesion nematodes recovered*	No. of lesion nematodes observed	No. of observed lesion nematodes with <i>Pasteuria</i>	Proportion of lesion nematodes with <i>Pasteuria</i>	Source of specimen
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Survey of *Pasteuria* on *Pratylenchus* in organic farm soils:

MD2B	5	5	1	20.0%	Soil
PA11B	4	4	3	75.0%	Soil
PA15C	2	2	1	50.0%	Soil
PA16	20	10	2	20.0%	Soil
PA17A	5	5	1	20.0%	Soil
MD2A	4	4	0	0.0%	Soil

Baiting trial to increase detection of *Pasteuria*:

PA17A	4	4	2	50.0%	Corn roots
MD2A	40	40	4	10.0%	Corn roots
MD2A	16	16	2	12.5%	Bean roots

* Total number lesion nematodes recovered for sample PA16 is extrapolated from a partial sample count.

Table 5.3. Mean population density of *Pratylenchus* recovered from certified organic farm soils in the Mid-Atlantic states.

	No. <i>Pratylenchus</i> /100 cm ³ soil	No. of Samples
Samples without <i>Pasteuria</i> on <i>Pratylenchus</i>	10.1 ± 4.9	16
Samples with <i>Pasteuria</i> on <i>Pratylenchus</i>	6.7 ± 2.7	6

* Mean ± standard error.

Table 5.4. Correlation coefficients of soil parameters measured for 16 of 22 samples in which *Pratylenchus* was detected in a survey of organic soils from five Mid-Atlantic states. *Pasteuria* was not detected on *Pratylenchus* in these samples.

	<i>Pratylenchus</i>	pH	CEC	P ₂ O ₅	K	Ca	Mg
<i>Pratylenchus</i>	1.00						
pH	0.56	1.00					
CEC	0.38	ns	1.00				
P ₂ O ₅	0.35	ns	0.57	1.00			
K	ns	ns	0.56	0.47	1.00		
Ca	0.47	0.46	0.96	0.54	0.43	1.00	
Mg	ns	ns	0.79	0.59	0.71	0.72	1.00

* soil pH (pH), cation exchange capacity meq/100g (CEC), and pounds per acre of available phosphorus pentoxide (P₂O₅), potassium (K), calcium (Ca), and magnesium (Mg).

Table 5.5. Correlation coefficients of soil parameters measured for 6 of 22 samples in which *Pasteuria* was detected on *Pratylenchus* in a survey of organic soils from five Mid-Atlantic states.

	<i>Pratylenchus</i> + <i>Pasteuria</i>		pH	CEC	P ₂ O ₅	K	Ca	Mg
<i>Pratylenchus</i>	1.00							
<i>Pratylenchus</i> + <i>Pasteuria</i>	ns	1.00						
pH	-0.83	-0.39	1.00					
CEC	ns	ns	0.36	1.00				
P ₂ O ₅	-0.67	0.37	0.57	ns	1.00			
K	ns	ns	ns	0.64	ns	1.00		
Ca	-0.57	-0.40	0.79	0.83	ns	0.62	1.00	
Mg	0.41	ns	ns	0.32	ns	-0.43	ns	1.00

* soil pH (pH), cation exchange capacity meq/100g (CEC), and pounds per acre of available phosphorus pentoxide (P₂O₅), potassium (K), calcium (Ca), and magnesium (Mg).

5.2 Increasing detection of *Pasteuria*

Pasteuria were detected on *Pratylenchus* recovered from plant roots for samples MD2A and PA17A (Table 5.1 and Table 5.2). The calculated number of lesion nematodes per green bean plant ranged from two to 30.6 individuals, with an average of 15.5 nematodes in five samples. Average lesion nematode density on corn was 154.7, with the number of individuals per sample ranging from zero to 452.8 for six samples. The green bean planted for sample PA17A died, so data are not available for this treatment.

5.3 Reproduction of *Pr. penetrans* in four soil treatments

The number of lesion nematodes recovered from treatments ranged from none to 467 per gram root (Figure 5.3). Differences between container types were not significant, so data were combined to test for effects of the four soil treatments. The number of *Pratylenchus* nematodes differed significantly ($P = 0.001$) among soil treatments. Paired contrasts (Table 5.6) indicated that higher lesion nematode densities were present in uninoculated soil treatments than in the sterile or non-sterile soil treatments that were inoculated with *Pr. penetrans*. Indigenous *Pr.*

crenatus were recovered from uninoculated soils and may have been the source of high lesion nematode populations.

Pasteuria endospores were observed on *Pr. crenatus* nematodes in one uninoculated control sample from a 250-cm³ glass beaker. *Mononchus* and *Helicotylenchus* were observed in one non-sterile treatment and *Pasteuria* endospores were found in the latter.

Figure 5.3. Means and standard error for the total number of lesion nematodes per 1-gram root from green bean (*Phaseolus vulgaris*) cv. Jade in four soil treatments.

Table 5.6. Paired contrasts of mean number of lesion nematodes recovered per gram root from green bean (*Phaseolus vulgaris*) cv. Jade in four soil treatments.

	Non-sterile soil	Sterile soil	90:10 Sterile:non-sterile soil
Untreated soil (Control)	P = 0.006	P < 0.0001	P < 0.0001
Non-sterile soil	-	P < 0.0001	P < 0.0002
Sterile soil	-	-	ns

5.4 Reproduction of *Pr. penetrans* in soils with and without *Pasteuria*

Soils that were not inoculated with *Pr. penetrans* had negligible recovery of lesion nematodes. Due to zero counts attributed to uninoculated treatments, data were log transformed ($\log_{10}[x + 1]$) to normalize the distribution. There were no significant differences between fresh and dry shoot weights of the bean plants at the 8-week harvest. Population densities of *Pr. penetrans* also did not differ significantly among soil types.

The presence or absence of *Pasteuria* endospores ($P = 0.03$) and time of harvest ($P = 0.0006$) significantly affected the number of lesion nematodes extracted (Table 5.7). Population densities were greater in pots where *Pratylenchus* inoculum was added, as expected, but did not differ significantly among other factors. Paired contrasts between soils in which *Pasteuria* were and were not previously detected on lesion nematodes and the time of harvest indicated that populations of *Pr. penetrans* decreased between the 2-week and 8-week harvest regardless of treatment (Table 5.8 and Figure 5.4). The mean population density in soils without *Pasteuria* was higher than in soils with *Pasteuria* at the 2-week harvest but this difference was not significant. At the 8-week harvest, nematode populations in soils without *Pasteuria* were significantly greater than in soils with *Pasteuria* ($P = 0.09$).

When included as a covariate in the statistical model, the effect of soil pH on lesion nematode densities was statistically significant, but the effect of *Pasteuria* remained significant (Table 5.9). Lesion nematode densities were higher in soils with higher pH. The average soil pH

for the 12 soils was 6.5, and differences between pH levels within each soil pair ranged from 0 to 0.5 (Table 4.2).

Pasteuria endospores were detected on three lesion nematodes, each from different samples. One nematode was identified as *Pr. penetrans* and the other two nematode individuals could not be identified. The endospore-attached *Pr. penetrans* was recovered from Sample PA12, and *Pasteuria* had not been detected in these soils. The unidentified lesion nematodes were recovered from Sample MD2B and PA15C. Endospore-attached nematodes were only detected in samples from the 2-week harvest.

Table 5.7. Analysis of variance of treatment effects on the number of lesion nematodes recovered per plant from different soil types with and without *Pasteuria* endospores.

Source	DF	Sum of Squares	F Ratio	P > F
<i>Pasteuria</i>	1	0.715434	4.7811	0.0313
Harvest	1	1.883756	12.5888	0.0006
<i>Pr. penetrans</i>	1	45.057656	301.1119	< 0.0001
Soil type	5	0.562728	0.7521	0.5866
Block	2	0.445268	1.4878	0.2311
<i>Pasteuria</i> * Harvest	1	0.007367	0.0492	0.8249
<i>Pasteuria</i> * <i>Pr. penetrans</i>	1	0.231201	1.5451	0.2170
<i>Pasteuria</i> * Soil type	5	0.868095	1.1603	0.3346
Harvest * <i>Pr. penetrans</i>	1	1.657656	11.0778	0.0012
Harvest * Soil type	5	0.132490	0.1771	0.9706
<i>Pr. penetrans</i> * Soil type	5	0.777273	1.0389	0.3995
<i>Pasteuria</i> * Harvest * <i>Pr. penetrans</i>	1	0.038351	0.2563	0.6139
<i>Pasteuria</i> * Harvest * Soil type	5	0.822695	1.0996	0.3660
<i>Pasteuria</i> * <i>Pr. penetrans</i> * Soil type	5	1.059712	1.4164	0.2255
Harvest * <i>Pr. penetrans</i> * Soil type	5	0.479773	0.6412	0.6688
<i>Pasteuria</i> * Harvest * <i>Pr. penetrans</i> * Soil type	5	1.005495	1.3439	0.2527
Error	94	14.065932		

Table 5.8. Paired contrasts of mean number of lesion nematodes recovered from soils at 2 and 8 weeks after inoculation in soil treatments with or without *Pasteuria* endospores.

Paired contrast	Significance (P-value)
2-week X 8-week, <i>Pasteuria</i> present	P = 0.01
2-week X 8-week, <i>Pasteuria</i> absent	P = 0.02
<i>Pasteuria</i> present X <i>Pasteuria</i> absent, 2-week	P = 0.17
<i>Pasteuria</i> present X <i>Pasteuria</i> absent, 8-week	P = 0.09

Table 5.9. Analysis of variance of treatment effects of soil pH on the number of lesion nematodes recovered from soils with and without *Pasteuria* endospores.

Source	DF	Sum of Squares	F Ratio	P > F
<i>Pasteuria</i>	1	0.574766	3.9881	0.0479
Harvest	1	1.883756	13.0707	0.0004
<i>Pr. penetrans</i>	1	45.057656	312.6391	< 0.0001
Block	2	0.445268	1.5448	0.2172
<i>Pasteuria</i> * Harvest	1	0.007367	0.0511	0.8215
<i>Pasteuria</i> * <i>Pr. penetrans</i>	1	0.231201	1.6042	0.2075
Harvest * <i>Pr. penetrans</i>	1	1.657656	11.5019	0.0009
<i>Pasteuria</i> * Harvest * <i>Pr. penetrans</i>	1	0.038351	0.2661	0.6068
Soil pH	1	0.606188	4.2061	0.0422
Error	133	19.168005		

Figure 5.4. The mean number of lesion nematodes recovered at 2 and 8 weeks after inoculation in soil treatments with or without *Pasteuria* endospores.

*Data are back transformed.

6.0 DISCUSSION

6.1 Survey of *Pasteuria* on *Pratylenchus* in certified organic cropping soils

Lesion nematodes were detected in 65% of soil samples collected from certified organic farms in the Mid-Atlantic United States. Endospores of *Pasteuria* were detected on *Pratylenchus crenatus* in 27% of samples in which lesion nematodes were observed. The mean population density of *Pratylenchus* in these soils was lower than in soils without *Pasteuria* on lesion nematodes. The damage threshold for *Pratylenchus* ranges from 50 to 1800 nematodes per 100 cm³ soil (Potter and Olthoff, 1993). Lesion nematode density was above the damage threshold in only one of the samples in which *Pratylenchus* occurred (PA14), and *Pasteuria* endospores were not detected in that sample. There were no clear indicators from the survey questionnaire or other analyses to explain the high densities observed in this sample compared to other samples.

Densities of *Pratylenchus* nematodes and *Pasteuria* reflect pre-season sampling used for this survey. Under ideal conditions, a survey of this nature would be based on post-season sampling as this is the time of year when lesion nematodes move out of plant roots and into surrounding soil. Densities of plant-parasitic nematodes tend to be lower in soils sampled prior to the crop-growing season compared to soils sampled near the end of the season (Bhan et al., 2010). It is possible that populations of lesion nematodes and, therefore, *Pasteuria* were below detection levels in some samples.

Kariuki and Dickson (2007) presented evidence that *Pa. penetrans* may be more readily detected from spring sampling because endospores are released from *Meloidogyne* cadavers after roots decay over the winter. Given that endospore-attached *Pratylenchus* were recovered from roots in the assays described in this report, endospore-filled lesion nematode cadavers may be present within plant roots.

Sample PA12 was used in the experiment to compare reproduction of *Pr. penetrans* in soils with and without *Pasteuria*. Although *Pasteuria* was not detected on the three *Pr. crenatus* recovered from the initial soil assay, *Pasteuria* was observed on a single *Pr. penetrans* recovered from a treatment that had been inoculated with *Pr. penetrans*. Occurrence of *Pasteuria* in this sample was not included in the survey results because it was detected as a result of incidental baiting. It is likely that a greater number of soils collected contained *Pasteuria* than were identified from soil extractions. Future surveys should bait all soils with target host nematodes to more accurately determine distribution and frequency of *Pasteuria*.

Kotcon (1990) collected 205 soil samples for a survey of plant-parasitic nematodes in West Virginia peach orchards between late March and early May 1986. Species of *Pratylenchus* were observed in 77% of the samples and the mean population density was 8.4 ± 0.7 lesion nematodes per 100 cm^3 soil. In a survey of plant-parasitic nematodes sampled from wheat and barley soils in early spring, Hollaway et al. (2008) detected lesion nematodes in 53% of samples and reported a median density of 9 nematodes per gram dry root. These results are similar to those found in this survey and show a similar pattern in frequency and distribution of lesion nematodes sampled in the spring. *Pratylenchus* occurred more frequently than all other plant-parasitic nematodes in assays from organic vegetable and cereal crop production soils in Germany (Hallman et al., 2007). These researchers collected both pre- and post-season soils, but results were not delineated based on the time of sampling.

The occurrence of *Pasteuria* on plant parasitic nematodes in Florida was reported by Hewlett et al. (1994). *Pasteuria* endospores were observed on nematodes recovered from soil and root samples that were assayed by the Florida Cooperative Extension Service from 1992 through 1993 and the Florida Department of Agriculture and Consumer Services, Division of

Plant Industry from 1990 through 1993. *Pasteuria* endospores were detected in 14 plant parasitic nematode genera collected from 41 Florida counties. *Pasteuria* endospores were reported on *Pratylenchus* in 21 of the 335 samples in which *Pasteuria* were observed on plant parasitic nematodes. Authors noted that samples submitted for these soil analyses did not represent an even distribution of soils in Florida. In this survey of certified organic cropping soils, additional plant parasitic nematodes were not targeted for analyses and observations of *Pasteuria* endospores on *Helicotylenchus* and *Hoplolaimus* were incidental.

Of the lesion nematodes with *Pasteuria* endospores attached, the number of endospores per nematode ranged from 1 to 10, with 1 to 2 endospores attached to 14 out of 15 specimens. In a survey of 73 samples collected from turf grass, 1 to 5 endospores of *Pasteuria* were attached to 64%, 66%, 70%, 78%, and 70% of *B. longicaudatus*, *H. galeatus*, *H. microlobus*, *T. annulatus*, and *Meloidogyne* specimens, respectively (Giblin-Davis et al., 1990). The total number of specimens observed for each genus ranged from 41 to 181, which supports recommendations to collect a greater number of samples for future surveys of *Pasteuria* on *Pratylenchus*.

Lesion nematode density was positively correlated with soil pH, calcium content, and cation exchange capacity in the 16 samples where *Pasteuria* was absent. In contrast, density of *Pratylenchus* was negatively correlated with soil pH, calcium, and phosphorus content in the six soils where *Pasteuria* was detected. These correlations could imply density dependent parasitism, and *Pasteuria* may have a greater effect on lesion nematode populations in soils that are conducive to *Pratylenchus*. These soil variables may affect the interaction between *Pasteuria* and *Pratylenchus* species; however, observed correlations could be a result of a statistical fluke since one or two outliers could have a disproportionate effect on correlations. The small sample size in this survey warrants additional studies to validate these findings.

Investigations of interactions between *Pasteuria* and physiochemical soil properties have been conducted previously (Afolabi et al., 1995; Mateille et al., 2002; Dabiré and Mateille, 2004; Dabiré et al., 2007). Soil texture and porosity appear to affect incidence of *Pasteuria* on plant-parasitic nematodes. In this study, soil type was not a significant factor influencing density of *Pratylenchus*. Soil textures in the survey samples ranged from fine to fine-loamy, fine-silty, and loamy-skeletal. Given the limited variation in type of soils sampled for this survey, it is not surprising that soil type was not correlated with differences in lesion nematode density in soils with or without *Pasteuria*. The majority of soils sampled for the survey had been carefully managed for organic crop production for several years. Ninety-two percent of the farm managers surveyed indicated use of high diversity crop rotation, 88% used green manure and cover crops, 80% used noncommercial manure, 64% used compost and/or compost extracts, and 52% used summer fallow to manage their crop growing soils.

Lesion nematode densities in soils with *Pasteuria* were negatively correlated with soil pH, with the mean pH being 6.5 in all data sets. Given the low number of survey samples, possible effects of soil pH on the interactions of *Pasteuria* and lesion nematodes cannot be deduced from these data. Nonetheless, Afolabi et al. (1995) measured the electrostatic potential (mV) of *Pasteuria penetrans* endospores at soil pH levels of 3, 7 and 9 in the presence of three different concentrations of lanthanum (La^{3+}), calcium (Ca^{2+}) and potassium (K^+). Endospores had the highest net negative charge at pH 7 with a potassium concentration of $10^{-5} \text{ mol l}^{-1}$. The electrostatic potential decreased as acidity or alkalinity increased. It also decreased at higher salt concentrations and cation valency. These authors indicated that endospore attachment to the nematode cuticle may be affected by electrolyte concentration and pH.

Chen et al. (1994) indicated a possible correlation between levels of inorganic nitrogen and density of *Pa. penetrans* infecting *Meloidogyne*. Densities of endospore-attached juveniles and infected females recovered from tobacco roots and field soils were higher in treatments amended with 158 kg ammonium nitrate/ha compared to those amended with 89 kg ammonium nitrate/ha. Analyses of soil organic matter were not conducted for all samples collected for this survey. Future studies should include assessment of nitrogen levels.

Mateille et al. (2002) detected *Meloidogyne* in 79.6% of 632 soil samples collected from six countries, and *Pa. penetrans* endospores were attached to juveniles in 36.8% of those samples. The proportion of root-knot nematodes with *Pa. penetrans* endospores attached was correlated with soil texture and densities of *Meloidogyne*. There was a positive linear correlation between *Meloidogyne* population densities and the proportion of endospore-attached juveniles. A similar trend was observed for soils with higher proportions of fine particles (clay and fine silt). The percentage of root-knot nematodes with *Pa. penetrans* decreased as the percent of coarse and fine sand particles increased. Soil textures for samples with *Pa. penetrans* varied from 1.5 to 30.2% clay and fine silt, and 30.1 to 91.2% fine and coarse sand. These authors did not indicate correlations between occurrence of *Pa. penetrans* on *Meloidogyne* and mean soil pH, which ranged from 6.3 to 7.3.

This study provides a baseline from which further investigations of *Pasteuria* as biological control agents of lesion nematodes in organic cropping systems may be conducted. The study would have benefited from collecting soil samples over a broader sampling area, increasing the number of samples collected, and sampling at the end of the growing season. Future researchers could conduct an initial assessment from a smaller volume of soil, such as a 1-liter sample instead of 19-liters, and then return to sites of interest to collect additional soil

needed to conduct experiments. Direct assessment of soil type in addition to using the USDA Natural Resources Conservation Service Web Soil Survey would provide a basis for a more detailed analysis.

Areas of further research include comparisons of density of *Pasteuria* on plant-parasitic nematodes collected from non-cultivated and cultivated (organic and conventional) soils. A long-term field experiment could be conducted to compare effects of different soil pH levels on the occurrence of *Pasteuria* on host nematodes in *Pasteuria*-infested soils. Experiments of this nature have not been conducted on *Pratylenchus*. The distribution and frequency of *Pasteuria* on lesion nematodes in many economically important crop-growing areas has not been reported. There is a critical need to assess occurrence of these organisms, and future surveys should target cropping systems with known susceptibility to *Pratylenchus*.

6.2 Increasing detection of *Pasteuria*

Populations of *Pasteuria* have been augmented on host nematodes in field soils and greenhouse pot cultures as described above (Section 2.1.4). Soils were selected based on the hypothesis that *Pasteuria* was present but had not been observed due to low recovery of lesion nematodes from samples. *Pasteuria* was previously detected on lesion nematodes recovered from samples collected from the same farm. In this test, *Pasteuria* endospores were observed on lesion nematodes recovered from roots for Sample MD2A. The MD2A soil sample was collected from a cropping area adjacent to the site where MD2B soils were collected and where *Pasteuria* was previously detected on *Pr. crenatus*. Sample MD2A was the only soil in which *Pasteuria* was detected on lesion nematodes as a result of this baiting test. Presence of *Pasteuria* on lesion nematodes was confirmed for Sample PA17A.

Lesion nematodes were extracted from plant roots but not from rhizosphere soil. In natural conditions, endospore attachment is known to occur only in the soil matrix; although, Mankau and Imbriani (1975) suggested endospore attachment could occur within a plant root. *Pasteuria* endospores remain attached after the nematode enters a plant root, and endospore-attached nematodes could have migrated out of roots during extraction. Hewlett and Dickson (1993) induced attachment of *Pasteuria* endospores to nematodes by centrifugation of nematodes in a suspension of endospores. It is possible that endospores were released from nematode cadavers during shaker extraction and may have attached to other nematodes in the suspension of water and roots.

Recovery of endospore-attached nematodes from roots may suggest that cadavers degrade inside roots and release endospores that attach to lesion nematodes migrating out of roots after hatching. Alternatively, nematodes may have acquired endospores while migrating through soil, entered the roots, and re-emerged with the endospore still attached before the *Pasteuria* fully colonized the host nematode. Either explanation may be worth investigating to determine if endospore-infested roots may be cultivated as a source of *in vivo* inocula for *Pasteuria* species infecting vermiform endoparasites, such as *Pratylenchus*.

6.3 Reproduction of *Pr. penetrans* in four soil treatments

This experiment was conducted to determine if the nature of lesion nematode suppression in the WVU Organic Farm soils is general or specific. The expected outcome was that lesion nematode reproduction would be lowest in the non-sterile field soils compared to the sterilized soil and that suppressiveness would re-establish in the 90:10 sterile:non-sterile treatment.

The population density decrease in the sterile and 90:10 sterile:non-sterile soil treatments compared to a 50% population density increase in the non-sterile soil treatment indicated that

either sterilization of the field soil was deleterious to nematode reproduction, or indigenous nematode inoculum overwhelmed the *Pr. penetrans* inoculum added to the sterile and non-sterile soil treatments. *Pratylenchus penetrans* were recovered from all four treatments and *Pr. crenatus* were recovered from all treatments except for the sterile soils.

Nematode density in the uninoculated control treatment was significantly higher than in all other treatments. Due to a misunderstanding, this treatment was added to the bench 4 weeks after the three experimental treatments. Plants were allowed to grow for an 8-week period, and during the last 4 weeks of growth, these containers did not have other plants to compete with on the lab bench. It is likely that observed outcomes from the control treatment do not accurately reflect differences between the inoculated and uninoculated treatments.

Treatment effects would have been measured more effectively if data were collected for all response variables, including number of nematodes per 100 cm³ soil and total plant root weight. Analyses of total nematode density per plant, as opposed to density per 1-gram root, would provide stronger evidence to support observed outcomes.

Additionally, results may have been affected by the density of nematode inoculum. Densities of nematodes recovered from WVU greenhouse pot culture were lower than expected. The presence of the predatory nematode *Mononchus* in the *Pratylenchus* pot culture may have affected these nematode populations. However, *Pr. penetrans* often is difficult to maintain in greenhouse pot culture (personal observation).

Mankau (1975) measured effects of *Pa. penetrans* soil treatments on tomato plants in greenhouse pot culture. Parameters measured were plant height, number of leaves, root-gall rating, and the dry weight of roots and shoots. The soil treatments were (1) air-dried endospore-infested soil, (2) sterilized endospore-infested soil, and (3) air-dried field soil without

endospores. Soils were steam sterilized at 15 psi for 1 hour to kill the endospores. For each treatment, four 10-cm plastic pots were filled with soil and a 2-week-old tomato seedling was transplanted into each pot. After 11 days, 10,000 *M. incognita* J2s were inoculated into each pot and data were collected 70 days after inoculation. Tomato plants grown in air-dried endospore-infested soil had a significantly higher number of leaves and dry shoot weight compared to the other two soil treatments. The gall rating for plants from endospore-infested soils was 8.5, which was significantly lower than the rating of 20.5 for sterilized soils and 22.7 for field soils. The results of this experiment indicate that steam-sterilization deactivates endospores, yet effects of steam-sterilization were not significantly different from untreated field soils.

Weibelzahl-Fulton et al. (1996) conducted a greenhouse assay using four soil treatments to determine if suppression of *Meloidogyne incognita* and *M. javanica* was caused by *Pa. penetrans*. The four soil treatments were: autoclaving, microwaving, air-drying and untreated field soils. The amount of root galls, egg masses, and eggs were significantly lower in the microwaved, air-dried, and untreated soils compared to the autoclaved soils.

Suggestions to improve this assay include recovery of nematodes from soil and root samples, and measuring root and shoot weights. A higher density of *Pr. penetrans* inoculum would improve detection of differential reproduction of lesion nematodes. Assessment of other biological antagonists present in these soils is also warranted. Future experiments could use alternative soil sterilization methods in conjunction with chloropicrin to eliminate *Pasteuria* endospores from sterile treatments.

6.4 Reproduction of *Pr. penetrans* in soils with and without *Pasteuria*

Pasteuria were detected on *Pratylenchus* species in six soils collected during a survey of certified organic farm soils. These soils were used to compare reproduction of *Pratylenchus*

penetrans in soils with and without *Pasteuria* endospores present. Results of the experiment that compared reproduction of *Pr. penetrans* in four soil treatments showed that varying levels of background *Pratylenchus* populations could interfere with treatment effects. To reduce indigenous lesion nematode populations without adversely affecting *Pasteuria* endospores, the soils used for this experiment were heated in a drying oven at 66 C. Results for this experiment indicate that this dry-heat treatment effectively reduced background *Pratylenchus* populations. Endospore-attached *Pratylenchus* were recovered at the 2-week harvest and the dry-heat treatment did not appear to hinder *Pasteuria* endospore attachment. *Pratylenchus penetrans* were recovered from four samples that were not inoculated with lesion nematodes, and this species was not detected in two of these samples prior to this assay.

Cetintas and Dickson (2004) compared suppression of root-knot nematodes by *Pa. penetrans* in four soil treatments. Soils were autoclaved, microwaved, air-dried or untreated. Differences among root galling in treatments that were not inoculated with root-knot nematodes were not significant; however, tomato roots from microwaved soils had higher levels of root galling than roots from the untreated control and autoclaved soil treatments. No root galls were observed on tomato roots from the air-dried soil treatments. Although soil temperatures after microwaving were approximately 75°C, this treatment did not fully eliminate the background population of root-knot nematodes in these soils. To ensure background nematode populations are eliminated in future assays, soils should incubate for more than 22 hours at a minimum temperature of 75°C. Soils should also be assayed to determine presence of microbes assumed to be eliminated by heat treatment.

At the 2-week harvest, populations of *Pratylenchus penetrans* were lower in soils with *Pasteuria* than without *Pasteuria*, but differences were not significant. The same trend was

observed for the 8-week harvest, and these differences were statistically significant. Mankau (1975) reported densities of *Pr. scribneri* on *Phaseolus vulgaris* recovered after 55 days were reduced by more than 50% for soils with *Pa. penetrans* compared to soils without *Pa. penetrans*. This finding supports results observed for *Pr. penetrans* in this experiment.

Endospores have been reported to attach and germinate within 24 hours of nematode exposure to *Pasteuria*-infested media for juveniles of *Meloidogyne*, *Heterodera* and *R. reniformis* (Chen and Dickson, 1998; Bird et al., 2003; Davies, 2009; Hewlett et al., 2010; Schmidt et al., 2010; Davies et al., 2011). Lesion nematodes inoculated into *Pasteuria*-infested soils may have become infected within the first two weeks after inoculation, making it likely that the level of endospore inoculum in soils with *Pasteuria* increased during the 8-week growing period.

Studies of suppression of plant parasitic nematodes by *Pasteuria* have variable results. Cetintas and Dickson (2004) suggested that augmentation of *Pa. penetrans* would be necessary to maintain suppression of root-knot nematodes in field soils. Some studies show *Pa. penetrans* is suppressive to root-knot nematodes in long-term cropping systems (Bird and Brisbane, 1988; Chen et al., 1994; Mankau, 1980; Minton and Sayre, 1989). However, Atibalentja et al. (1998) reviewed variable results obtained from multiple studies (Spaull, 1984; Ciancio et al., 1992; Giblin-Davis et al., 1990; Verdejo-Lucas, 1992; Ciancio, 1995). Whether naturally occurring populations of *Pasteuria* can maintain lesion nematode densities below the damage threshold in cropping soils is unknown. There is a critical need to develop formulations of *Pasteuria* for use as inundative nematode control.

Plants were harvested at 2 weeks post-inoculation to allow nematode populations to become established in roots but before significant reproduction would occur. Soil factors may

have caused nematode mortality and reduced the density of nematodes available for extraction at the 2-week harvest. It may be necessary to assess initial population densities at 7 to 10 days post-inoculation.

In assays conducted for the survey of organic farm soils, *Pasteuria* endospores were observed on *Pratylenchus crenatus*. This was the only lesion nematode species confirmed in four of these six soils. *Pasteuria* endospores were observed on lesion nematode juveniles and one unidentified *Pratylenchus* for two soils. In some cases, a species or isolate of *Pasteuria* parasitizes only one nematode species because of strict host preference for a nematode race and even a single population (Stirling 1985). It is possible that the *Pasteuria* isolate(s) present in these soils may only parasitize *Pr. Crenatus*, and infection of the *Pr. penetrans* inoculum would be negligible.

Pasteuria was observed on a *Pr. penetrans* specimen recovered from a soil where *Pasteuria* had not been previously detected. *Pasteuria* in this sample may have been specific to *Pr. penetrans* and was not detected due to lack of this lesion nematode species in that soil before inoculation. This observation supports the need to collect soils at the end of the growing season, when *Pasteuria* endospores are most prevalent and more likely to be detected on host nematodes. Additionally, the soil bioassay method described by Oostendorp et al. (1990) and Weibelzahl-Fulton et al. (1996) could be used to determine the presence of *Pasteuria* in soils where it was not previously detected.

Lesion nematode densities, as well as occurrence of *Pasteuria* on plant-parasitic nematodes, vary with soil type (Norton, 1978; Kotcon, 1990; Afolabi et al., 1995; Mateille et al., 2002; Dabiré and Mateille, 2004; Dabiré et al., 2007). Population densities of *Pr. penetrans* in soils with or without *Pasteuria* were not affected by soil type in this experiment. Factors

discussed in Section 6.1 may be attributed to this observation. The 12 soils assayed had one of four soil textures: four samples were fine textured, five were fine-loamy, one was fine-silty, and two were loamy-skeletal. Effects of soil type may be better assessed by increasing sample size and by confirming soil types through direct assessment as well as the USDA NRCS Web Soil Survey.

This experiment could have been improved by increasing the density of lesion nematodes in the inocula, collecting samples from pre- and post-season soils, and by examining for the presence of *Pasteuria* endospores on nematodes when fewer than 20 individuals were recovered from soil. Naturally occurring populations of the host nematode and *Pasteuria* could be augmented by baiting soils as previously described. Endospore-filled lesion nematodes could be recovered from soils using methods described by Oostendorp et al. (1991b). *Pasteuria* endospores could be collected from infected cadavers and then mixed into soils to increase endospore loads; however, this is a tedious process, especially for small vermiform nematodes such as *Pratylenchus*.

7.0 CONCLUSIONS

Experiments in this study assessed the distribution and frequency of *Pasteuria* endospores and *Pratylenchus* nematodes in certified organic cropping soils from the Mid-Atlantic region of the United States. These soils were used to compare reproduction of *Pr. penetrans* in soils with and without *Pasteuria* endospores, as determined by their presence on lesion nematodes.

Pratylenchus species were present in 65% of soil samples collected from certified organic farms, and *Pasteuria* endospores were detected on *Pratylenchus crenatus* in 27% of samples in

which lesion nematodes were observed. Population densities of lesion nematodes were lower in soils with *Pasteuria* than in soils where *Pasteuria* had not been detected.

Soils baited with plant host crops to increase detection of *Pasteuria* resulted in detection of *Pasteuria* endospores on *Pr. crenatus* in two soil samples. In one experiment, *Pasteuria* was observed on *Pr. crenatus* recovered from untreated WVU Organic Research Farm soils. In another experiment, *Pasteuria* was detected on *Pr. penetrans* from a soil sample that had been inoculated with *Pr. penetrans*. Endospore-attached lesion nematodes were recovered from plant roots for three out of four soils, and *Pasteuria* had not been previously observed on *Pratylenchus* from two of these soils.

To the best of the author's knowledge, this is a first report of *Pasteuria*-like endospores attached to *Pratylenchus crenatus* from Maryland, Pennsylvania, and West Virginia and to *Pratylenchus penetrans* from Pennsylvania. Also, endospores characteristic of *Pasteuria* are reported on *Hoplolaimus* sp. from Pennsylvania and on *Helicotylenchus* sp. from Ohio.

An analysis of reproduction of *Pr. penetrans* on *Phaseolus vulgaris* cv. Jade in four soil treatments failed to determine if suppression of nematodes in these soils is caused by a biological agent because background indigenous nematode populations obscured results. Two different volumes of soil (400 cm³ and 150 cm³) and two types of planting containers (plastic pot and glass beaker) yielded similar densities of lesion nematodes, indicating equivalent compatibility for nematode reproduction. These findings were used to determine the amount of soil and type of planting container needed to conduct the experiment that compared reproduction of *Pr. penetrans* in soils with and without *Pasteuria*.

In the experiment to evaluate reproduction of *Pratylenchus penetrans* on *Phaseolus vulgaris* cv. Jade in soils with and without *Pasteuria* endospores, populations of *Pr. penetrans*

were lower in soils with *Pasteuria* and differences in final population densities were significant at $P = 0.05$. Results support the hypothesis that the mean rate of increase of *Pr. penetrans* populations infecting *Phaseolus vulgaris* cv. Jade will be lower in soils containing *Pasteuria*-attached *Pratylenchus* nematodes than in soils where *Pasteuria* was not detected. When soil pH was included as a covariate in the statistical analyses, densities of *Pr. penetrans* increased with higher pH levels; however, the effect of *Pasteuria* remained significant.

In summary, observations from this research indicate that *Pasteuria* is present on *Pratylenchus* in organic cropping soils, indigenous *Pasteuria* can be baited to increase its detection in soils, endospore attachment can be observed on lesion nematodes recovered from plant root extractions, and reduction of *Pr. penetrans* populations is greater in soils with *Pasteuria* than in soils without *Pasteuria*. Continued research into methods to enhance this biocontrol activity will be needed before *Pasteuria* will produce reliable lesion nematode control in commercial agriculture.

8.0 APPENDIX A

Host crop history for soils collected from certified organic farms in the Mid-Atlantic states.

Sample ID	Date soil collected	Host crop(s) at sampling	Cover crop(s) Fall 2007/Winter 2008	Rotational crop(s) Summer 2007
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Samples with *Pratylenchus*, with *Pasteuria* detected on *Pratylenchus*

MD2A	03/24/2008	Millet	Hay, rye	Grain crops
MD2B	03/24/2008	Rye-Clover	Hay, rye	Grain crops
PA11B	04/06/2008	Sweet corn	Vetch, winter rye	Mixed vegetables
PA15C	05/14/2008	Garlic	None	Mixed vegetables
PA16	05/14/2008	Corn	Wheat	Mixed vegetables
PA17A	04/07/2008	Corn	Varieties unknown	Mixed vegetables

Samples with *Pratylenchus*, without *Pasteuria* detected on *Pratylenchus*

MD3A	03/24/2008	Radish	Clover, vetch, oats	Mixed vegetables
MD3B	03/24/2008	Brassicas	Clover, vetch, oats	Mixed vegetables
MD22	06/06/2008	Potato	Varieties unknown	Corn
MD23	06/06/2008	Clover, orchard grass	Oats	Corn
MD24	06/06/2008	Strawberry	Varieties unknown	Mixed vegetables
OH5	05/15/2008	Green bean	Varieties unknown	Mixed vegetables
OH6A	05/16/2008	None - fallow	None - fallow	None - fallow
PA11A	04/06/2008	Green bean	Vetch, winter rye	Mixed vegetables
PA12	04/06/2008	Hay	Hay	Hay
PA14	04/06/2008	Brassicas	Varieties unknown	Mixed vegetables
PA15A	05/14/2008	Garlic	None	Mixed vegetables
PA17B	04/07/2008	Tomato	Varieties unknown	Mixed vegetables
PA17C	04/07/2008	Rye	Varieties unknown	Mixed vegetables
PA19	05/14/2008	Hay, clover	Winter wheat	Hay, clover
PA20B	05/14/2008	Potato	Vetch, white clover	Mixed vegetables
WV25	06/06/2008	Pea	Rye, buckwheat	Mixed vegetables

Samples without *Pratylenchus*

MD1	03/24/2008	Tomato	Vetch, rye	Mixed vegetables
MD4	03/24/2008	Tomato	Vetch	Mixed vegetables
MD21	06/06/2008	Vetch, orchard grass	Rye	Mixed vegetables
OH6B	05/16/2008	Hay, clover	Hay, clover	Hay, clover
OH7	05/15/2008	Brassicas	Varieties unknown	Mixed vegetables
OH8	05/15/2008	Rye	Varieties unknown	Mixed vegetables
OH9	05/15/2008	Garlic	Winter wheat	Mixed vegetables
PA10	04/07/2008	Brassicas	Rye, oats, buckwheat	Mixed vegetables
PA15B	05/14/2008	Mixed vegetables	None	Mixed vegetables
PA18	05/14/2008	Bean, pea, corn	Vetch, rye	Mixed vegetables
PA20A	05/14/2008	Brassicas	Vetch, white clover	Mixed vegetables
VA26	06/07/2008	Pea	Varieties unknown	Mixed vegetables

Mixed vegetables may include the following crops: tomato, corn, peppers, potato, beans, Brassica crops, lettuce, greens, garlic, pea, soybean, cucumber, grains, eggplant, radish, squash.

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