OFFICIAL

COOPERATIVE MULTI-STATE RESEARCH PROJECT
NORTHEAST REGION

PROJECT NO.: NE-171 (Revised)
TITLE: Biologically Based IPM Systems for Management of Plant-Parasitic Nematodes
DURATION: October 1, 1999 to September 30, 2004

STATEMENT OF THE PROBLEM:

Modern agriculture continues to rely on technological advances and off-farm inputs to increase yields and profitability. In particular, most crops rely heavily on fertilizers and pesticides to maintain acceptable production levels.

Plant-parasitic nematodes are important pathogens on most food and fiber crops and without appropriate controls will cause loss of yield and quality. Chemical nematicides have been the primary management tool for over fifty years but many products have now been removed from the market or are under review. There is an urgent need for the development of alternative control options. Such work is necessary to develop environmentally sound agricultural systems that minimize chemical use while maintaining high production standards.

JUSTIFICATION:

Most crops are susceptible to damage by plant-parasitic nematodes and without effective management profitable production could not continue. Conventional controls rely heavily on chemicals, genetic resistance or crop rotation. However, nematicides and fumigants have frequently been targeted as human or environmental hazards and the future of many of these compounds is uncertain. For example, methyl bromide will be removed from the market. In President Clinton's proposed budget for FY2000, 5 million dollars are allocated for a methyl bromide transition program. Pesticide manufacturers have set nematicide research and development as a low priority due to the costs associated with registration and the high risks associated with product development. Genetic resistance and crop rotation have limitations as stand-alone controls. Resistant varieties have been developed for only a few crops and these must be used judiciously to reduce the selection pressure for races able to break nematode resistance. Rotation alone is limited by logistics and requirements of specific crops, economic feasibility and the host range of the problem nematode species.

Given the diverse crops and production systems in the Northeast, a multi-state research project on major crops of importance to the region is desirable because it facilitates information exchange and sharing of methods more readily than traditional single state or single crop projects. By sharing details of what works and especially what doesn't, information on improved nematode control tactics can be disseminated regionally and across cropping systems more rapidly than would otherwise occur through research publications. Thus, a regional approach is likely to benefit growers more quickly as well as being more likely to lead to broader theoretical advances that cut across disciplines and cropping systems. The focus on turf and crops selected for study, such as peaches, carrots, potatoes, strawberries, onions, and lettuce, are economically important in the Northeast. There are crop losses due to nematode infestations that need attention.

This research will bring nematode management in line with national initiatives to implement IPM methods in major crop production. Integrated control tactics will be developed

around biological interactions that work in concert with crops and reduce dependence on chemicals. Further funding opportunities will be explored to enhance this multi-state research effort. This approach will ensure that agriculture in the twenty-first century is both economically viable and ecologically more sustainable. The objectives of NE-171 address IPM and sustainable agriculture and have been identified as high priorities for national research by the Joint Council on Food and Agricultural Sciences, the Experiment Station Committee on Organization and Policy and the U. S. Department of Agriculture.

RELATED CURRENT AND PREVIOUS WORK:

Crop rotation, soil amendments, genetic resistance:

Nematode control tactics such as rotation, cover cropping, green manuring, organic amendments and plant resistance have been evaluated as alternatives to chemical nematicides. Crop rotation is perhaps the oldest and most effective cultural practice for controlling plant-parasitic nematodes (Good, 1968). Rotation crops that reduce nematode populations have been shown to serve as either nonhosts, less suitable hosts, or as antagonistic plants (Johnson, 1982; Trivedi and Barker, 1986; Merwin and Stiles, 1989; Halbrendt, 1996). Nonhosts may simply not be parasitized and have an effect similar to fallow. Antagonistic plants may produce compounds which stimulate hatch without allowing reproduction or actively reduce nematode populations by the production of nematicidal or toxic compounds (Halbrendt, 1996). Nematode-suppressive plants which produce these compounds may be utilized to deliver them by means of rotation, inter-cropping, cover cropping, or soil amendment with either green manure or dry plant residue.

A number of rotation or cover crops which reduce nematodes more than fallow have been identified (Halbrendt, 1996; Ko and Schmitt, 1996; LaMondia, 1997; Rodrìguez-Kàbana, 1992). Crop rotations which include these antagonistic or allelopathic plants may be thought of as active and those that are simply non-hosts as passive (Rodrìguez-Kàbana, 1992). The list of plants antagonistic to nematodes has grown but research on the mechanism of allelopathy, resistance or antagonism has not kept pace. While an understanding of the mechanism involved is not necessary for control by either resistance or allelopathy, this information would be important for selection and development of breeding lines with increased efficacy.

Two promising grain crops for nematode reduction by rotation are diploid 'Saia' oats (*Avena strigosa*) and sorgho-sudangrass (*Sorghum bicolor x S. sudanense*). These crops have been reported to be poor hosts of lesion and root-knot nematodes (Colbran, 1979, Fay and Duke, 1977). Grain or grass rotations and companion crops conserve soil, reduce compaction and increase water infiltration into soil (Newenhouse and Dana, 1989). The use of oats and sorghum as allelopathic rotation or companion crops can suppress a number of weed species (Neustruyeva and Dobretsova, 1972, Putnam and DeFrank, 1983). Some oat accessions exuded up to three times as much scopoletin, a root growth inhibiting compound, as standard 'Garry' oats (Fay and Duke, 1977). Oats and sorghum also have been shown to produce fungicidal root exudates toxic to soilborne fungi such as *Fusarium* and *Gaumannomyces* (Crombie and Crombie, 1986, Nimbal et al., 1996). Recently, resistance to lesion nematodes has been associated with greater production of avenocin in 'Saia' oats than in susceptible oats (B. B. Brodie, pers. comm.).

Because of the importance of developing non-chemical nematode controls, a number of antagonistic plants have recently been bred for increased efficacy against nematodes and other soilborne pathogens. These include selected varieties of *Brassica* spp and *Tagetes* spp.

The nematode-suppressive effects of marigold (*Tagetes* spp.) have been documented for over 50 years (Tyler, 1938; Steiner 1941). The antagonistic effect is due to toxicity of metabolites in the root exudates (Siddiqui and Alam, 1987a, b; 1988a). Although the presence of nematicidal compounds has been well documented, there are conflicting reports concerning the efficacy for suppression of a number of nematodes and the compounds responsible (Gommers et al., 1980; Gommers and Bakker, 1988). The differential ability of *Tagetes* species to suppress nematodes may be due to variation in species, cultivars, edaphic factors, nematode populations, and the active nematicidal compounds (Eisenback, 1987; Arevalo and Ko, 1989; Alam et al., 1990). Recently, marigold cultivars have been specifically selected for increased nematicidal efficacy.

A number of leguminous crops also have been reported to have nematode suppressive properties. Vicente and Acosta (1987) reported that velvetbean root exudates were nematicidal and Rodriguez-Kàbana (1992) suggested that bacteria in the velvetbean rhizosphere were antagonistic to cyst and root-knot nematodes.

Other crops such as canola (rapeseed, *Brassica napus* L. and *B. campetris* L), also have been implicated in reducing soil densities of nematodes (Davis et al. 1993; Mojtahedi et al. 1993). These plants synthesize large quantities of sulfur-containing glucosinolates in all tissues (Sang et al. 1984). When these plants are incorporated into the soil as a green manure, the glucosinolates hydrolyze to fungicidal and nematicidal isothiocyanates (Ettlinger et al. 1968). The level of nematode control has been correlated with glucosinolate concentration in plant extracts (Jing and Halbrendt, 1994). The incorporation of canola shoots to soils heavily infested with *Meloidogyne chitwoodi* reduced nematode populations more than fallow treatments (Mojtahedi et al. 1993), and the application of canola meal significantly reduced populations of *P. neglectus* and subsequent Verticillium wilt in potato (Davis et al. 1993).

Additional alternative practices include soil amendments and mulches. Green manure treatments with certain nematode antagonistic plants may provide even greater nematode control than when used solely as a cover or rotation crop. (Siddiqui and Alam, 1987c, 1988b). Composts have been widely used as peat substitutes in the nursery industry, resulting in disease suppression (Hoitink and Grebus, 1994). The mechanisms put forth for this suppression (Alam and Jairajpuri, 1990) have included: changes in the physical and chemical properties of soil, including nutrition and the production of nematotoxic substances released directly or by microbial breakdown; changes in the microbial ecology of soil that affect antagonistic organisms or the release of antagonistic microbial metabolites; and the induction of plant resistance or tolerance to nematodes as well as the induction of systemic acquired resistance (Zhang et al, 1996; Zhou and Paulitz, 1994). LaMondia et al. (in press) demonstrated over 4 years in field microplot studies that soil amendment with spent mushroom compost reduced potato early dying severity by 24% (AUDPC for symptomatic leaves), increased marketable tuber yields by 94%, increased leaf photosynthesis by 43%, and reduced lesion nematode densities in subsequent rye cover crops by 48%.

Plant resistance is the most economical and environmentally safe means of controlling plant-parasitic nematodes. Host plant resistance and tolerance are important when crops must be grown in the presence of potentially damaging populations of nematodes. Resistance refers to the suppressive effects of plant genes on nematode development and/or reproduction (Trudgill, 1991). Tolerance refers to the ability of a plant to grow and produce an acceptable yield while supporting moderately large numbers of plant parasitic nematodes. Ideally, plant resistance and tolerance could be combined.

Most host plant resistance has been identified for specialized host-plant relationships, such as the sedentary endoparasitic cyst and root-knot nematodes, but resistance to other nematodes such as the migratory endoparasite *Pratylenchus*, also has been identified (Potter and Dale, 1994). Mechanisms of resistance to nematodes include both constitutive plant compounds and factors induced by nematode infection. Resistant plants may contain toxic compounds such as alpha terthienyl, present in marigolds (Veech, 1981), compounds which reduce egg hatch (Gapasin, 1988), or lectins which may affect host-finding ability (Marban-Mendoza et al., 1992). Incompatible host response to nematodes ranges from nonspecific tissue necrosis to necrosis around a feeding site or a hypersensitive response that prevents nematode development (Kaplan and Keen, 1980).

Genes conferring resistance have been identified and incorporated into many crop cultivars. Resistance to root-knot and cyst nematodes in vegetable crops and tobacco have been identified and developed as a part of this project (Thies et al. 1997; LaMondia, 1991). While resistance has been durable in many situations, selection by resistance genes has often resulted in a population shift to nematode races able to overcome plant resistance. It will be important to integrate resistance with other control tactics to maintain nematode control and reduce selection pressure against resistance gene(s).

Biological control agents:

Suppression of plant-parasitic nematodes with nematode predators, parasites or disease agents is a desirable alternative to chemicals. Deploying and managing biocontrols will likely become increasingly important components of integrated pest management programs and sustainable agricultural systems. Biological control agents occur in diverse taxa and include nematode trapping or endoparasitic fungi, predatory nematodes, arthropods (e.g. mites and collembola), bacterial parasites, and predatory protozoa. Understanding this diversity will be a critical step in adapting management practices to realize the full potential of biological control. However, because of the large number of potential biocontrol agents it is desirable and beneficial to focus efforts on one organism.

Pasteuria penetrans is a promising biological control agent against root-knot nematodes in the southeastern United States and has been selected for detailed studies. The role of *P. penetrans* in the northern tier of states has not been evaluated. The use of this bacterium to suppress plant-parasitic nematodes has been tested on many crops, mostly in greenhouse pots (Chen and Dickson, 1998). *Pasteuria penetrans* suppressed *Meloidogyne* spp. on bean, brinjal, chickpea, cucumber, eggplant, grape, hairy vetch, kiwi, mung, okra, peanut, pepper, rye, soybean, tobacco, tomato, and wheat (Chen and Dickson, 1998). Some isolates of *Pasteuria* spp. have been reported to suppress *Belonolaimus longicaudatus* on bermudagrass (Giblin-Davis, 1990), *Heterodera avenae* and *H. zeae* on unspecified crops (Bhattacharya and Swarup, 1988), *H. cajani* on cowpea (Singh and Dhawan, 1994), *H. elachista* on rice (Nishizawa, 1987), and *Xiphinema diversicaudatum* on peach (Ciancio, 1995b).

While many strains of *Pasteuria* are nematode species-specific, cross-generic suppression of nematodes also has been observed (Mankau and Prasad, 1972; Bhattacharya and Swarup, 1988). *Pasteuria penetrans* simultaneously reduced population densities of *Pratylenchus scribneri* and root galls induced by *M. javanica* and *M. incognita* in tomato (Mankau and Prasad, 1972). An Indian isolate of *P. penetrans* parasitized both *Heterodera* spp. and *M. incognita* (Bhattacharya and Swarup, 1988). Endospores of *P. penetrans* were mass-produced on *M.*

incognita and when endospores were incorporated into soil, numbers of cysts of *H. avenae* on wheat roots were reduced.

A successful example of the biological control potential of *P. penetrans* for management of root-knot nematodes on peanut was reported recently (Chen, 1996; Chen et al., 1996). Endospores of *P. penetrans* were incorporated into field microplots in the first year only at 0, 1,000, 3,000, 10,000, or 100,000 endospores/g of soil. Root galls and pod galls were significantly reduced at 100,000 endospores/g of soil in the first year. In the second year, root galls and pod galls were reduced at 10,000 and 100,000 endospores/g of soil. Pod yields increased 58% and 94% at 10,000 and 100,000 endospores/g of soil, respectively (Chen et al., 1996). In the third year, root galls and pod galls were nil at 100,000 endospores/g of soil, and were reduced at 1,000, 3,000, and 10,000 endospores/g of soil. Pod yields were increased 180%, 291%, 221%, and 272% at 1,000, 3,000, 10,000, and 100,000 endospores/g of soil, respectively (Chen et al., unpubl.). Population densities of J2 in soil at harvest also were significantly reduced at 10,000 and 100,000 endospores/g of soil in the third year. Apparently, the establishment and amplification of *P. penetrans* in the field microplots played an important role in the increased suppression of root-knot nematodes over the 3-year period.

Isolates of *Pasteuria* spp. failed to suppress populations of *Meloidogyne* spp. on sugarcane (Spaull, 1984), *Helicotylenchus lobus* on turfgrass (Ciancio et al., 1992), and *Tylenchulus semipenetrans* (Ciancio and Roccuzzo, 1992). A survey in sugarcane fields in South Africa revealed that population densities of *Meloidogyne* spp. were generally higher in fields infested with *P. penetrans* and that the level of nematode parasitism was greater at higher nematode densities (Spaull, 1984). On turfgrass, there was no correlation between the population density of *Helicotylenchus lobus* and the percentage of nematodes with endospores (Ciancio et al., 1992). However, an increase in parasitism was observed 2 months after a 10-fold nematode population growth (Ciancio et al., 1992).

Mode of action: Pasteuria penetrans reduced the number of J2 penetrating roots (Brown and Smart, 1985; Davies et al., 1988a; 1988b; Sekhar and Gill, 1990), number of females in roots (Davies et al., 1991), female fecundity (Bird, 1986; Bird and Brisbane, 1988), number of J2 in soil (Chen et al., 1997c; Davies et al., 1988a; 1988b), and number of eggs on roots (Ahmed and Gowen, 1991; Bird and Brisbane, 1988; Chen et al., 1997c; Weibelzahl-Fulton et al., 1996). Movement and mobility of J2 were reduced and their ability to locate host roots was affected when J2 were encumbered with endospores (Davies et al., 1991; Mankau and Prasad, 1977).

Pasteuria species are gram-positive, dichotomously branched, endospore-forming bacteria with septate mycelium (Mankau and Imbriani, 1975). Most of the species identified to date show great promise as biological control agents of several of the most important plant-pathogenic nematodes. To date four species of the bacterium have been described. These were differentiated by their host preference, developmental characteristics, and size and shape of sporangia and endospores (Sayre and Starr, 1989). *Pasteuria ramosa,* which parasitizes water fleas of the genera *Daphnia*, is the type species of the genus (Ebert et al., 1996). The other three species of *Pasteuria* are parasites of plant-parasitic nematodes: *P. penetrans* on *Meloidogyne* spp., *P. thornei* on *Pratylenchus* spp., and *P. nishizawae* on cyst nematodes of the genera *Heterodera* and *Globodera* (Sayre and Starr, 1989). The terminal hyphae of the mycelium elongate to form sporangia and eventually endospores. Endospores are nonmotile and resistant to desiccation.

The apparent utility of *P. penetrans* for biological control of root-knot nematodes has prompted several efforts to produce the organism in culture (Bishop and Ellar, 1991; Riese et al.,

1988; Previc and Cox, 1992). At present, only limited production of *Pasteuria* has been attained *in vitro*. Major hurdles to the development of *Pasteuria* spp. as a biological agent include the definition and control of events necessary for the formation of infective spores. In the absence of protocols to mass produce spores of *Pasteuria* spp. for direct application, the development of strategies for their amplification via agronomic practice still holds great promise for the biocontrol of target nematode species (Dickson, unpublished).

In order for spores to attach to juvenile root-knot nematodes, they must bear appropriate surface molecules that recognize and bind to receptors on the cuticle of the nematode host. Interference with spore attachment by lectins specific for N-acetylglucosamine and/or alpha-glucoside, alpha-mannoside residues suggests these ligands may participate in the recognition process (Bird et al., 1989). Proteins isolated from *P. penetrans* spores have been shown to react with wheat-germ agglutinin (WGA) and concanvalin A (ConA), which indicates the presence of potential ligand receptors on the spores. These findings have provided the basis for a model in which glycopeptides on the surface of the spores, designated as spore adhesins, are recognized by lectins on the cuticle of the nematode (Davies and Danks, 1993; Persidis et al., 1991). Antibodies directed against these adhesion proteins as well as the proteins themselves were able to inhibit attachment of the spores to the nematode cuticle (Charnecki et al., 1996; Davies et al, 1992; Davies and Danks, 1993, Davies and Redden, 1997; Persidis et al., 1991).

The surface coat of plant-parasitic nematodes carries a net negative charge (Spiegel and McClure, 1995). The spores of *P. penetrans* also bear an electronegative charge that is affected by pH and ionic strength, as well as ion valency (Afolabi et al., 1995). The pH and ionic composition of a medium will be considered as variables in performing attachment assays to characterize potential adhesions. More recent studies have implicated that hydrophobic forces also participate in the overall adhesion process (Davies et al., 1996). It is important to note that this overall process may be complex, with several different types of chemical interactions participating. The interaction of specific polypeptides with specific ligands may be most important in the recognition process as an initial step in the overall adhesion process.

Polyclonal antibodies have been prepared that react with several spore-specific proteins and which block the attachment of spores to the host cuticle (Charnecki et al., 1996; Chen et al., 1997b). These will be used to identify peptide and/or carbohydrate epitopes in specific proteins involved in the recognition processes. Also, two mouse monoclonal antibodies have been produced, one (2A4 IgM) which detects an epitope shared by several peptides resolved by SDS-PAGE, and the other (5F1) which detects an epitope on a single polypeptide. Both of these monoclonal antibodies are able to discriminate different spore isolates with respect to native surface antigens (detected by ELISA) and to denatured polypeptides (detected by Western Blots following SDS-PAGE).

The 2A4 IgM antibody was produced in ascites and purified by gel filtration. It detects a number of bands ranging in size from 23 to 205 KDa by Western blots. These were resolved by SDS-PAGE of endospore extracts, and it is probable that it recognizes a glycan epitope. The ability of the lectin, wheat-germ agglutinin (WGA), to detect these same bands indicates that they bear glycan moieties including either b(1-4)-linked N-acetyl gluosamine or sialic acid residues. The inability of the 2A4 monoclonal antibody to detect fetuin, ovalbumen or pancreatic ribonuclease B, all of which are readily detected with WGA, indicates that this antibody recognizes an epitope that includes but is not restricted to the glycans recognized by WGA. (Charnecki et al., 1998). Using ELISA with plated endospores from different isolates (P20, P120,

and B4) indicate that these epitopes are on the surface of the spores. This same antibody failed to react with plated *Bacillus subtilus* endospores (evaluated by ELISA) or with UDC extracts of these spores (evaluated by Western blot), which indicates that they are relatively unique to endospores of *Pasteuria* spp. (Harrison and Preston, unpublished).

Spores have been developed that can be fluorescently labeled with fluorescein isothiocyanate with no detectable loss of their ability to attach to second-stage juveniles (J2) of *M. incognita* (Charnecki et al., 1996). The labeling involves the conjugation to spore coat proteins different from most of those detected with antibodies that block the attachment of spores to nematodes. These labeled spores also are able to infect nematodes to provide progeny spores. These have been particularly useful in providing quantitative data on the ability of various ligands to block attachment of endospores to nematodes. With this approach, the 2A4 monoclonal antibody was shown to effect 50% inhibition of the attachment of P20 spores to *M. arenaria* race 1 at an IgM concentration of 1.3×10^{-10} M. This antibody and its $(Fab')_2$ and Fab fragments will be those first used for the isolation and characterization of the adhesin epitope(s).

The primary approaches to biological control of nematodes have been augmentation of indigenous control agents and inundation with specific organisms. Long rotations or monoculture of susceptible hosts can induce microflora suppressive to specific nematode pests that maintains the pest population at levels below economic thresholds. The best known example is the widespread control of *Heterodera avenae* in Europe by the fungi *Nematophthora gynophila* and *Verticillium chlamydosporium*. In the US, suppression of *Meloidogyne* spp. by the bacterium *Pasteuria penetrans* has been reported (Weibelzahl-Fulton, et al., 1996). Augmentation of indigenous agents also has been proposed as a mode of action for a variety of organic soil amendments (Rodriguez-Kabana, 1986; Kaplan and Noe, 1993).

Inundative release of biocontrol agents has met with success in small plots (Hewlett, et al. 1998), but commercial successes in larger field-scale trials have not yet been achieved (Duncan and Noling, 1998). Major constraints include limited understanding of the ecological niche requirements for introducing organisms to soil and the cost of producing large quantities of the most fastidious parasites of nematodes. In most cases, the agents being considered for release are highly specialized for effectiveness against particular nematode species. For example, the bacterium *Pasteuria penetrans* occurs in a variety of races that differ in their ability to attack different species of nematodes. Attachment of the bacterium to the host appears to be a key step in regulating this specificity, with different strains attaching preferentially to different nematode species (Oostendorp, et al., 1990). Research into this and other aspects of the activity of this agent is difficult because of the obligate parasitic nature of *Pasteuria*, and little success has been achieved in attempts to grow the bacterium in pure culture.

One tactic to suppress nematodes with the potential for low-cost, broad application is the use of endophytic fungi in grasses. Endophyte-infected fescue significantly reduced reproduction of *Meloidogyne marylandii* and *Pratylenchus scribneri* (Kimmons, et al., 1990) while an endophyte-infected ryegrass supported significantly fewer *Xiphinema americanum* than an endophyte-free cultivar (Dernoeden, et al., 1990). Because this agent is seed-borne, its use may be easily integrated into cropping systems using grasses as cover or rotation crops, however, a better understanding of the mode of action and the conditions promoting maximum efficacy is needed.

Integrated Pest Management:

Integrated Pest Management systems are a subset of cropping systems practices. These include integration of crop rotations, cover crops, organic amendments, tillage practices, crop protection practices (physical, chemical and biological), and economic and environmental considerations.

At its simplest, IPM programs for nematodes involve evaluating pest density relative to a damage or treatment threshold. Integration of genetic resistance may occur through adjusting the damage threshold to account for specific differences in tolerance among varieties or by suppressing nematode reproduction to maintain populations below the damage threshold. Integration of biological and cultural controls may involve either some measure of the biological control efficacy against the target pest, or implementation of recommended management practices that are known to reduce pest densities while simultaneously providing other cropping system benefits (e.g., rotations, organic soil amendments). Evaluation of these recommended management practices usually involves monitoring changes in nematode population densities. Although this empirical approach lacks the theoretical value of a mechanistic understanding, in many cases, it is the only practicable approach for assessing highly complex soil systems.

Crop rotations can reduce nematode population densities, but may increase populations of other nematode pests, particularly in multi-species nematode communities (Noe, 1998). Integration must also consider economic returns of rotations crops, additional capital and labor requirements, and grower and market acceptance of the rotation crop. In the Northeast, rotations are already used as part of management programs for *Globodera rostochiensis* on potato (Brodie, et al., 1993; Mai and Lownsberry, 1952), *Heterodera schachtii* on sugar beet (Mai and Abawi, 1980), *Meloidogyne hapla* on carrot (Kotcon, et al., 1985) and *Xiphinema* spp. on peach. While crop rotations alone are unlikely to provide nematode control in all nematode-crop systems, their potential as one component of IPM systems has not yet been fully realized. This potential, when integrated with other non-chemical management measures, is likely to become increasingly important as traditional nematicide options are lost.

Biocontrol agents span a continuum between generalist polyphagous organisms and specialists adapted to a single nematode host species or even a limited number of nematode host races. Augmentation of indigenous communities often implicitly relies on a broad diversity of biocontrol agents, of which each is adapted to differing seasons, host life stages, and environmental conditions in the soil. This high biodiversity is needed to assure that at least some of the agents present in a field will be active against the right nematode stages at the right time to promote adequate levels of population suppression. Because of this complexity, understanding the roles and relative importance of individual agents in any particular field is difficult and more progress has been made in systems dominated by one or two highly effective specialists. An ideal agent for commercialization would have a relatively broad spectrum of activity against plant-parasitic nematodes and would be able to successfully colonize a variety of soil environments and cropping systems, but would simultaneously avoid environmental risks from significant non-target effects (Simberloff and Stiling 1996). The indigenous nature of agents such as *Pasteuria penetrans*, as well as the limited dispersal ability of both these agents and their host nematodes, are factors that demonstrate the inherent ecological safety of biological control as a nematode control strategy.

The patchy distribution and soil-borne nature of nematodes, coupled with the high cost of monitoring and the need to make nematode management decisions well in advance of planting, are

special constraints to nematode IPM which emphasize the importance of additional research efforts to support nematode IPM (Duncan and Noling, 1998). Improvements in biological monitoring methods are needed to better assess the spatial distribution of plant-parasitic nematodes as well as the presence of biocontrol agents that may influence nematode population dynamics and yield loss relationships (Sikora, 1992).

Long-term sustainability based on a rigid management system is difficult in the face of a rapidly changing economic environment, yet long-term commitments are needed to achieve the benefits of many cultural and biological control practices (Noe, 1998, Duncan and Noling, 1998). The solution to this paradox requires a range of management options that can be implemented as short-term economic conditions dictate, while still integrating the long-term agroecological "costs and benefits" to the cropping system so that the true worth of particular management practices is adequately considered. Multi-year modeling efforts that integrate population dynamics, environmental variables, yield loss relationships, efficacy of management measures and economic factors have been touted as essential to optimize nematode management, but the research base to support such predictive models is lacking in all but a few cropping systems (Duncan and Noling, 1998). Thus, the challenge of sustainable nematode IPM programs is to understand the multifaceted impacts and interactions of multiple management tactics in order to allow growers to make optimum decisions for long-term nematode control while maximizing economic returns.

With the assistance of Mr. Alan Moore of USDA/CRIS, a search of the USDA Research (CRISTEL database) has been conducted for the field of plant nematology using the following key words and phrases: field crops (potatoes, carrots, lettuce, onions), fruits (peaches and strawberries), and integrated pest management. One hundred and forty-five studies were identified. There are other multi-state research projects (W-186, S-282 and NC-215) in the United States that address plant nematode problems, but these projects have different objectives and focus on other crop systems (e.g., alfalfa, field corn, sweetpotato, okra, cotton, wheat, soybean and melon). The proposed NE-171 project outline does not duplicate the efforts of other research projects to date. Comments by peer reviewers support this conclusion.

OBJECTIVES:

Objective 1: Evaluate the effects of rotational crops, organic amendments and host crop genetics on nematode community structure.

Objective 2: Characterization of biological control agents for suppression of plant-parasitic nematodes.

Objective 3: Comparison and evaluation of IPM system management of plant-parasitic nematodes based on crop rotation, organic amendments, host crop resistance and biological control agents.

PROCEDURES:

All state and federal laws will be followed in securing the required permits for interstate movement of pathogens, nematodes, soil or other regulated items.

Objective 1: Evaluate the effects of rotational crops, organic amendments and host crop genetics on nematode community structure.

Agricultural systems in the Northeast are diverse and often specific to certain locations or markets. As a result, nematode pathogens may be as diverse as the specialized crop systems they

attack. Our research involves both direct collaboration and complementary studies by individual researchers designed to extend results from model systems for wider applicability.

Scientists in eight states will conduct complementary or collaborative studies to assess the impact of rotation and cover crops (CT, MA, MD, MI, NY, PA, RI, and WV) on plant-parasitic nematode populations and nematode community structure. Results from our current project have identified several nematode-antagonistic crops. These crops include rotation and cover crops such as marigold, velvetbean, sesame, crotalaria, *Avena strigosa*, sudangrass and sorgho-sudangrass. These crops will be evaluated for nematode suppressive effects against root-knot, cyst, lesion or dagger nematodes in parallel (CT, PA) or complementary (NY, WV) studies. The mechanism of antagonism of cover and rotation crops, which reduce populations of these nematode genera during growth or as a green manure, will be determined in the greenhouse, field microplots, and in the field by scientists in CT, NY, and PA. The toxicity of root exudates or plant breakdown products such as glucosinolates released by the breakdown of *Brassica* residues (Sang et al 1984) or the nematicidal residues from oats, sudangrass or sorgho-sudangrass will be evaluated *in vitro* or directly on nematodes in soil to determine the most efficacious use against particular nematodes. Data and techniques developed in complementary systems will be shared to allow us to evaluate, compare, and increase efficacy of antagonistic plants on different nematodes. For example, the release of cyanogenic compounds and suppression of root-knot nematodes by sudangrass was increased when plants were incorporated prior to the first frost in NY. While juveniles were unaffected, egg hatch was reduced by exposure to plant infusions. A low volume soil bioassay technique developed in PA will be used to further evaluate the toxicity of green or freeze-dried plant extracts on a variety of plant parasitic nematodes using soils and nematodes supplied by cooperators in CT, MA, NY, and WV.

The effects of organic soil amendments on nematodes will be investigated in CT, MA, MI, NY, PA, RI, and WV. The mechanism of suppression will be evaluated by determining the direct effects of amendments against nematodes (CT, MI, NY, PA, and WV) in soil assays as well as the indirect effects of amendments on microbial populations such as the growth of bacterial species (RI), which produce fatty acids toxic to nematodes. Genetic plant resistance developed in CT, MD and ARS-SC as well as heritage varieties with resistance or tolerance (WV), will be evaluated for potential integration with nematode antagonistic crops. The effects of these treatments on nematode diversity and community structure will be assessed and evaluated as a potential indicator of soil health. Diversity and maturity indices will be developed in WV and MI to assess the effects of nematode control tactics on community structure.

Objective 2. Characterization of biological control agents for suppression of plant-parasitic nematodes.

Obj. 2.1) Survey plant-pathogenic nematodes for occurrence of *Pasteuria* spp.

A workshop will be conducted during the first multi-state meeting of the new project to teach members how to recognize *Pasteuria* spp. on different species of plant-parasitic nematodes. A pamphlet will be prepared and distributed to each member of the technical committee for use as a guide for proper handling of *Pasteuria* spp. The workshop and pamphlet will ensure that all members are following prescribed methods in our goal to document the occurrence of *Pasteuria* on plant-parasitic nematodes in northern regions of the United States. The following protocol will be followed. Sites chosen for sampling will be based on former cropping history and the importance of plant-parasitic nematode diseases. Extension personnel will be contacted for input

regarding the sites chosen. Priority will be given to identifying important agricultural sites that were formerly conducive for nematode disease, but have become or are becoming suppressive for the nematode disease. If conducive-suppressive sites can not be identified, then sites will be chosen that have a known high density of plant-parasitic nematodes. Soil and root samples will be collected from the chosen field sites based on a sampling pattern described by Barker et al., 1986. The samples will be stored at 10 C until they are processed (Barker et al., 1986). Nematodes will be extracted from soil and roots by methods described by Barker et al., 1986). The presence or absence of *Pasteuria* spp. will be documented, the nematode host identified, the soil classified, and the crop plant, and degree of crop damage associated with the nematode recorded. If *Pasteuria* species are present, the bacterium will be stored in a sand medium for further study. Cooperating states are FL, MD, MI, NY, and PA.

Obj. 2.2) Determine the survivability and host preference of isolates of *Pasteuria* spp. from different geographic regions.

Numerous isolates of *Pasteuria* spp. have been collected from field sites in Florida that have been identified as being suppressive to root-knot, lesion, ring, and sting nematodes. Also, an isolate of *Pasteuria* spp. was recently reported on the soybean cyst nematode, *Heterodera glycines*, in Illinois (Noel and Stanger, 1994). Selected isolates from Florida and those identified under objective 2.1 from northern states will be compared for their survivability, host preference, and biocontrol potential. Florida isolates that attach to *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* will be tested on *M. hapla*, the northern root-knot nematode and indigenous populations of *M. incognita*. Preliminary trials have shown that one Florida isolate of *P. penetrans* attached to MD populations of *M. incognita* and *H. glycines*. Selected isolates of *Pasteuria* spp. will be increased in the greenhouse on their preferred host. Once sufficient inoculum is produced, the parasite will be introduced into root-knot nematode infested microplots in the field. Both the nematode and its parasite will be monitored over time to determine whether the parasite will increase to suppressive levels and whether it will survive the winter season. Cooperating states are FL, MD, and MI.

Obj. 2.3) Evaluate different crops and methods for growing *Pasteuria penetrans*.

Various plants will be evaluated for production of *P. penetrans* endospores. Plants to be evaluated include tomato, squash, eggplant, cucumber, radish, okra, and pumpkin. The plants will receive single or double inoculations with nematodes that have endospores of *P. penetrans* attached. Characteristics to be evaluated include (but are not limited to) host suitability, ease of culture, ease of endospore recovery and endospore yield.

Similarly, novel culture techniques will be evaluated for efficiency of producing large quantities of *P. penetrans* endospores. Comparisons will be made between standard greenhouse pots, a growth chamber set-up equipped with a unique soil moisture control system recently reported by Sardanelli and Kenworthy (1997), and a hydroponic system located at The Land Pavilion located at Walt Disney World, EPCOT . Cooperating states are FL and MD.

Obj. 2.4) Determine the sequence of events required for the formation of *P. penetrans* endospore-associated proteins (adhesions) required for the attachment of spores to the cuticle of nematode hosts.

The biochemical events that occur during the development of *P. penetrans* are poorly understood. Research on spore adhesion may provide valuable insight into the unique relationship the bacterium has with its nematode host. The sequence of events required for the formation of spore-attachment proteins (adhesions) will be studied. Two *P. penetrans* isolates will be cultured

on *Meloidogyne arenaria* race 1 and *M. incognita*, respectively, and increased on tomato. IgM monoclonal antibodies (Mab) directed against spores from these two isolates that specifically recognize spore adhesions will be used to probe their formation as a function of development. Twelve, 16, 24, and 38 day old healthy and *P. penetrans* infected females will be extracted from roots. Proteins will be extracted with SDS and separated by PAGE. Gels will be electro-blotted on nitrocellulose membranes and proteins probed with the Mab. Cooperating states are FL and NY.

Obj. 2.5) Morphological and phylogenetic analysis of a *Pasteuria* sp. from ring nematode.

An isolate of *Pasteuria* sp. discovered in ring nematode, *Mesocriconema ornata* will be studied by electron microscopy to determine its morphology and also subjected to analysis by molecular techniques. The phylogenetic position of this species relative to that of *P. penetrans* and *P. ramosa* will be determined based on analysis of the 16S rDNA sequence encoding 16S ribosomal RNA. These sequences will be amplified by PCR and compared with sequencing reported for two isolates of *P. penetrans* (Anderson, et al, 1998). Cooperating states are MI and FL.

Obj. 2.6) Additional studies.

The influence of organic amendments as sources of organic acids, as well as specific compounds such as propionic and butyric acids on microbial communities will be characterized in CT and RI. The hypothesis being tested is that bacteria and other microorganisms produce organic acids under anaerobic conditions in which biocontrol activity is stimulated.

Population dynamics of predatory nematodes will be compared to plant-parasitic nematode dynamics under various rotation sequences in WV. Several methods to assess the role of nematode trapping fungi will be compared to identify methods that best characterize indigenous biocontrol activity. Another series of experiments will examine the role and mode of action of tall fescue infected with endophytic fungi (e.g., *Neotyphodium coenophialum*) for its potential as a rotation or cover crop to enhance nematode suppressiveness in orchard crops.

Objective 3. Comparison and evaluation of IPM system management of plant-parasitic nematodes based on crop rotation, organic amendments, host crop resistance and biological control agents.

Ten states (CT, FL, MA, MD, MI, NY, PA, RI, SC and WV) will be involved in the comparison and evaluation of IPM system management of plant-parasitic nematodes based on crop rotation, organic amendments, host crop resistance, and biological control agents. These studies will range from narrowly focused efforts targeted at reducing pest densities below specific damage thresholds, to broad multi-disciplinary analyses of whole farming systems. Results of procedures under objective 1 and 2 may, in some cases, be extended directly to this objective, whereas in other cases, additional specific trials will be needed to evaluate promising tactics in the context of overall IPM programs.

Integrated management of potatoes for control of root lesion and root knot nematodes and potato early dying using compost, green manure, and crop rotations in nematode IPM systems will be assessed in microplots and field plots (MI, CT, WV). While these studies will largely be conducted in association with other on-going projects, collaboration through use of comparable treatments under this regional project will extend the application of the results more broadly across the Northeast. Integration of nematode-resistance and biological control agents in

pepper will be evaluated (SC, MD) using agents screened under Objective 1. Collaboration with NY using *Pasteuria penetrans* and CT and MD using *Verticillium lecanii* will involve field trials of promising isolates against root knot and cyst nematodes.

Farming systems research in Michigan will evaluate nematode community structure in four farming systems (conventional, integrated fertilizer, integrated compost, and organic) as part of the Long Term Ecological Research Site. Similar studies of farming in West Virginia will include organic farming systems evaluations of orchard, vegetable, and field crop production operations. These studies will emphasize non-chemical nematode controls via rotations and soil amendments (animal manures versus green manures). Economic analyses of these practices will be conducted under related projects.

Although efficacy of these practices for nematode control can be inferred by changes in nematode population densities, biocontrol agent population dynamics and nematode community structure will be analyzed to provide a better mechanistic understanding of the effects of these systems and the opportunities for their application to IPM programs.

DESCRIPTION OF COOPERATION:

While much of the interdependence of this project may be evident from the procedures as outlined above, the cooperation between researchers in the project may be summarized as follows:

Nematode-suppressive crops identified in CT and NY will be evaluated for toxicity to nematodes using an assay developed in PA. The effects of nematicidal exudates and plant breakdown products on biological control agents and soil ecology will be evaluated in CT, NY, PA, RI and WV using techniques developed in FL. Biological control potential in soil, especially for *Pasteuria*, will be determined based on standard methods developed in FL and demonstrated to the group during a workshop on the topic which will be held at the first multi-state technical committee meeting of the project.

Host plant resistance developed in SC will be evaluated in root-knot nematode management systems in CT and MD.

The comparison and evaluation of tactics developed in individual states will be tested for applicability on different cropping systems throughout the region. Systems approach research will be done in MI and WV to evaluate these practices for nematode control and total nematode community structure.

EXPECTED OUTCOMES:

Objective 1. Commercially acceptable levels of nematode control will be achieved by the integration of multiple tactics such as rotation, cover cropping and soil amendments with genetic host resistance, where available. The integration of host resistance with soil amendments, rotation and cover crops will increase the overall efficacy of nematode control, as well as extend the effective utility of resistance. Nematode control techniques based on this approach will provide ancillary benefits to agricultural production such as erosion control, nutrient trapping and improved soil structure.

Objective 2. Improved methods of collecting and culturing biocontrol agents such as *Pasteuria* will facilitate larger scale experiments which are necessary to evaluate the feasibility of field applications. Knowledge of the host range of potentially useful biocontrol agents is essential to assess their value in IPM programs to control specific nematode problems.

Objective 3: Genetic resistance, cropping systems and biological controls will be integrated into nematode management programs that can be readily applied at the farm level. Comparisons of individual strategies with integrated management systems will identify those strategies that can be widely adopted, as well as those that are successful only in some regions or only as part of a multiple strategy combination.

ORGANIZATION:

The technical committee will consist of at least one voting member from each of the participating states (Attachment A), the administrative advisor, and the CSREES representative. Each year the technical committee will elect a chairperson, secretary, and at least one member-at-large to serve as an executive committee. The regional technical committee will meet annually to report on the research results obtained, discuss and exchange information and ideas and to plan and coordinate next year's work relating to the objectives of this proposal. A coordinator for each of the objectives may be designated to facilitate the coordination and reporting of the research being conducted by the collaborators. The technical committee may invite other scientists with experience in biological control, crop production systems, integrated pest management, sustainable agricultural practices, and others to participate in the annual meeting to provide specific information and strengthen the discussion.

SIGNATURES

Regional Project: Biologically Based IPM Systems for Management of Plant-Parasitic Nematodes NE-171 (Revised)

Administrative Advisor — Date: February 9, 1999

Chairman, Regional Association Directors — Date: 3/9/99

Administrator, CSREES — Date: 5/13/99

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ATTACHMENTS:

Table #1
A. Project Leaders and Participating Institutions
B. Resources
C. Critical Review
D. List of Publications
E. Response to Reviewers

Table 1. State or agency participation by crops, nematodes, and objectives

State	Crops	Nematodes	Objectives
Connecticut	Strawberry Potato	*Pratylenchus spp.* *Meloidogyne spp.*	#1, #2, #3
Florida	Vegetables: (eggplant, squash tomato, etc.)	*M. incognita* *M. arenaria* *H. glycines*	#1, #2, #3
Maryland	Vegetables Soybeans Corn	*M. incognita* *H. glycines* *H. zeae*	#1, #2
Massachusetts	Tomato	*M. incognita*	#1, #2, #3
Michigan	Potato Soybean	*P. pentrans* *H. glycines*	#2, #3
New York (Ithaca) (Geneva)	Vegetables: (onion/lettuce) Cabbage/Table beet Bean/pea	*M. hapla* *H. schachtii* *Pratylenchus spp.*	#1, #2, #3
Pennsylvania	Fruits (apple/peach/sm. fruit)	*Xiphinema spp.* *Pratylenchus spp.*	#1, #2, #3
Rhode Island	Turf	*Hoplolaimus* *Tylenchorhynchus*	#1, #2
USDA/ S. Carolina	Vegetables: (Southern pea, sweet potato, okra, pepper)	*M. incognita*	#1, #3
USDA/ Maryland	Vegetables	*M. hapla*	#2
W. Virginia	Fruits Field crops	*Xiphinema spp.* *Pratylenchus spp.*	#1, #2, #3

Attachment A

State	Member	Specialization
Connecticut	J. A. LaMondia	Management, Allelopathy, Genetic resistance
Florida	D. W. Dickson	Biological control, Population dynamics, Management
Maryland	S. Sardanelli	Population dynamics, Resistance
Massachusetts	R. L. Wick	Management, Population dynamics, Biocontrol
Michigan	G. W. Bird	Cropping systems, Sustainability, Population dynamics, Modeling
New York (Geneva)	G. S. Abawi	Management, Population dynamics, Resistance, Biocontrol Allelopathy
New York (Ithaca)	J. Esnard	Biocontrol, Biochemical interaction
Pennsylvania	J. M. Halbrendt	Management, Allelopathy Virus vectors
Rhode Island	S. R. Alm	Biocontrol, Management,
USDA / S. Carolina	J. A. Thies	Resistance, Management
USDA / Maryland	S. L. Meyer	Biocontrol, Management
W. Virginia	J. Kotcon	Biocontrol, Management, Nematode - host interaction

ATTACHMENT B. RESOURCES

Participation in Northeastern Regional Research Project

Title: Biologically Based IPM Systems for Management of Plant-Parasitic Nematodes

ADMINISTRATIVE ADVISOR: Dr. L. Magnarelli
CSREES REPRESENTATIVE: Dr. R. Huettel

Planned resource commitments for participating institutions:

SAES/ Federal Institution	Project Leader	Professional Discipline	Annual Input SY	PY	TY
Connecticut	J.A. LaMondia	Nematology	0.6	---	0.20
Florida	D.W. Dickson	Nematology	0.2	0.2	0.30
	J.F. Preston	Biochem/ Microbiology	0.1	---	---
Maryland	S. Sardanelli	Nematology	---	1.0	---
Massachusetts	R.L. Wick	Nematology	0.2	0.5	0.85
Michigan	G.W. Bird	Nematology	0.1	0.1	0.25
	G.M. Garrity (consultant)	Microbiology	---	---	---
New York (Ithaca)	J. Esnard	Nematology/ Plant Path	0.6	---	---
New York (Geneva)	G.S. Abawi	Nematology/ Plant Path.	0.4	---	1.00
Pennsylvania	J.M. Halbrendt	Nematology	0.3	---	---
Rhode Island	S.R. Alm	Nematology	0.2	1.35	0.75
USDA/Maryland	S.L. Meyer	Nematology	0.1	0.10	0.10
USDA/S.Carolina	J.A. Theis	Nematology	0.1	---	0.20
West Virginia	J. Kotcon	Nematology	0.2	0.5	---
	Totals		3.0	3.75	3.65