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USE OF THE ENTOMOPATHOGENIC NEMATODE,
HETERORHABDITIS MARELATUS, TO CONTROL THE
COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA*

presented by

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Major Professor's Signature

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By

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ABSTRACT

USE OF THE ENTOMOPATHOGENIC NEMATODE, *HETERORHABDITIS MARELATUS*, TO CONTROL THE COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA*

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Colorado potato beetle (*Leptinotarsa decemlineata* Say) is an important pest of potato and has developed resistance to many insecticides. With current and future restrictions on pesticides, ecologically safe alternatives for Colorado potato beetle management are needed. The goal of this project was to develop *Heterorhabditis marelatus* (Liu and Berry), an entomopathogenic nematode, as a biological control alternative for Colorado potato beetle through understanding its pathogenicity, adaptation to field edaphic factors, most effective rates, and the optimum time of application. Under controlled conditions, *H. marelatus* survival and pathogenicity was highest in sand and sandy loam soil with water moisture levels between -0.4 and -0.001 MPa. In field plots naturally infested with Colorado potato beetle, treatments of 333 million, 667 million or 1 billion *H. marelatus*/m^2 of soil in 2000 and 2001 reduced beetle survival in contrast to control plots. Application timing (when 4th instar larvae were first present, peak of 4th instars or at peak pupation) did not significantly affect Colorado potato beetle survival but significantly more adults emerged in controls than in *H. marelatus* treated plots. Overall, the results suggest that *H. marelatus* has a potential for managing Colorado potato beetle under field conditions.

For Nicole

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INTRODUCTION

Colorado potato beetle, *Leptinotarsa decemlineata* (Say), feeding on potato foliage can cause significant yield loss in potato production in the United States. Under favorable conditions, larvae produced by overwintering adults and first generation adults and their larvae can cause up to 80-100% crop loss (Berry et al. 1997). This destructive ability and high levels of resistance in Colorado potato beetle to most available insecticides has led to the need for additional control tactics against this pest. Entomopathogenic nematodes provide an alternative to reliance on chemical insecticides. *Heterorhabditis marelatus* (Liu and Berry) is a relatively new species that shows promising potential for Colorado potato beetle control (Berry et al. 1997). Many studies have addressed the efficacy of entomopathogenic nematodes for control of Colorado potato beetle (Koppenhöfer et al. 1995, Kung et al. 1990). However, little is known about the effects of edaphic factors on the survival and pathogenicity of *H. marelatus* against Colorado potato beetle.

The goal of this project was to develop feasible control of Colorado potato beetle using entomopathogenic nematodes as part of an integrated pest management system for Michigan potato production through developing a research model that describes the interactions of Colorado potato beetle, environmental conditions, and the entomopathogenic nematode, *H. marelatus*. Specific objectives and hypotheses were:

Objective 1: To determine how soil type and soil moisture affect the pathogenicity and survival of *H. marelatus*.

Hypothesis 1: Soil type and moisture do not affect the pathogenicity and survival of *H.*

marelatus within soil.

Objective 2: To determine the most effective rates of *H. marelatus* to economically control Colorado potato beetle in the field.

Hypothesis 2: Pathogenicity does not differ based on varying rates of *H. marelatus*.

Objective 3: To determine the most suitable time of *H. marelatus* application for effective Colorado potato beetle control in the field.

Hypothesis 3: Time of application does not affect control of Colorado potato beetle by *H. marelatus*.

CHAPTER 1

LITERATURE REVIEW

Ecology of the Colorado potato beetle, *Leptinotarsa decemlineata*

Biology: Colorado potato beetle, *Leptinotarsa decemlineata* Say (Colorado potato beetle) overwinters as an adult and begins to emerge in the spring as the soil warms above its developmental threshold of approximately 10 °C (Weber and Ferro 1994). If a suitable host plant, usually a *Solanum* species, is available, Colorado potato beetle will begin to feed, mate and lay eggs. However, when there is no food source available, Colorado potato beetle must search by flights of up to 8-16 km. Therefore adequate flight performance is often very important to adult beetles and is likely selected for within populations (Weber and Ferro 1996).

Depending on climate Colorado potato beetle may have from one to three generations (Weber and Ferro 1994). After successful oviposition, eggs hatch in 4-8 d and the generation time from egg to adult can be as little as 21 d at temperature between 25 and 30 °C. Colorado potato beetle has four larval instars. At maturation, the fourth instar drops to the ground to pupate in the soil (Fig. 1.1) (Weber and Ferro 1994).

Distribution: Colorado potato beetle is native to North America. Its most likely origin is the Southwest United States and Mexico (Casagrande 1985). The primary host plants utilized by Colorado potato beetle (before the host switch to potato) were *Solanum rostratum* Dunal, *S. elaeagnifolium* Cavanilles, and *S. angustifolium* Miller (Weber and

Ferro 1996). Now Colorado potato beetle feeds on most *Solanum* species, including potatoes, eggplant and tomatoes. Colorado potato beetle is currently the most damaging insect pest of potato over much of the northern hemisphere.

Humans altered the distribution of Colorado potato beetle in the latter part of the 17th century. At that time, Colorado potato beetle was still feeding on *S. rostratum* (sand bur) and the burs from this plant easily attached to introduced cattle spreading throughout the western United States (Lu and Lazell 1996). With the increased distribution of its food plant came the increased distribution of Colorado potato beetle. This may explain collections of Colorado potato beetle over much of the western United States in the early 1800's and the confusion about the original distribution of Colorado potato beetle. According to Casagrande (1985), "The misconception about the discovery of the pest is not too serious, but not realizing it's Mexican origin was a serious mistake. Appropriate research in biological and cultural control might have avoided a century of reliance on chemicals and the widespread insecticide resistance that has resulted."

Host Plant Adaptations: Colorado potato beetle was in contact with potato as early as 1820; however, it did not make a host switch to potato until many years later. In fact, Colorado potato beetle was not a major crop pest until 1859 in the United States. Hsiao (1985) suggested that this host switch from *S. rostratum* to potato was due to a genetic change on one gene locus. This genetic transformation also seems to be associated with larvae color and it has been suggested that the gene or genes responsible for potato feeding had strong dominance with regard to feeding acceptance and oviposition preference (Lu and Logan 1994). This dominant trait could explain the

tremendous growth of the Colorado potato beetle population after 1859. By 1875, Colorado potato beetle was in such high numbers that it easily made the voyage to England in the same year. Colorado potato beetle rapidly spread to Europe and Asia and currently has a distribution that includes most of the northern hemisphere except part of eastern Asia and remote islands (Lu and Lazell 1996).

Abiotic factors have a major impact on survival and fitness of local populations of Colorado potato beetle. Some abiotic factors that cause beetle mortality in local populations are heavy rain (which kills early instars), extreme heat, extreme cold and wind. Nutritional quality of the host plants plays a major role in general health of Colorado potato beetle. Hunt et al. (1992) showed that Colorado potato beetle that fed on plants given high nitrogen fertilizer had significantly higher survival and faster maturation compared to beetles fed on plants with less nitrogen.

Biotic factors affecting Colorado potato beetle survival may include intraspecific competition and attack by natural enemies (Cappaert et al. 1991). With the spread of its food plant, Colorado potato beetle was able to make drastic changes in its habitat. This is especially true after the switch to potato. Colorado potato beetle originated in a dry desert environment, but its current distribution includes the Northern United States and Canada as well as most of Europe and countries of the former U.S.S.R. Colorado potato beetle can tolerate a wide range of environmental conditions and adapts rapidly to changes in its environment.

Ecological Factors: Competition among other species of insects and Colorado potato beetle is very limited because potato is toxic to most organisms. Leaves of

potatoes contain high amounts of glycoalkaloids that serve as a plant defense. Some aphids such as green peach aphid, *Myzus persicae* Sulzer (Aphididae, Homoptera) and the potato leafhopper, *Empoasca fabae* (Harris) (Cicadellidae, Hemiptera) are able to feed on potato foliage, but the level of competition between aphids, leafhoppers and Colorado potato beetle is generally minimal. Intraspecific competition by Colorado potato beetle, however, can occur at high population levels and result in decreased body weight and lipid reserves (Lucas et al. 1995). It is possible that this situation cycles through the years. If large females have greater fecundity than small females, then a year of small beetle populations could be followed by a year of large beetle populations and vice versa (Lucas et al. 1995).

Fecundity of Colorado potato beetle can be affected by host plant. Weber and Ferro (1996) showed that the amount and type of food given to adult female Colorado potato beetle affected both fecundity and flight performance. Low fecundity among the entire population of beetles could be detrimental to the local population. Usually large numbers of individuals ensure the survival of the population. But, when fewer eggs are laid, there is an increased chance of local extinction or bottleneck effect. After a bottleneck, a population may have difficulty rebounding to previous genetic diversity (Weber and Ferro 1996).

Decreased flight performance can be equally harmful to populations when host plant shortages occur. Females must have enough energy to fly to a suitable host plant for oviposition. Poor flight performance can result in female death without oviposition. Female beetles must often sacrifice high fecundity for increased resources allocated to fewer offspring, based on host plant. Colorado potato beetle can feed on any solanaceous

plant, but may prefer different species based on the geographic source of the potato beetle population (Cappaert et al. 1991, Hsiao 1978, Hsiao 1985).

Significance of *Leptinotarsa decemlineata* in Potato Production

Providing 15% of annual cash receipts, potato (*Solanum tuberosum* L.) is the most important vegetable crop in the United States, (Anon. 1999). As far as crops grown for human food use in the United States, potatoes rank second behind wheat in importance. The billions of dollars generated from the chipping and food service industries further enhance revenues generated by potato growers (Anon. 1999). In Michigan, potatoes have a retail value of $601,048,242 (Michigan Potato Industry Commission 1996).

Insecticides are the most commonly used means to control Colorado potato beetle, but there is an increasing problem of insecticide resistance (Grafius 1997, Georgiou and Lagunes-Tejeda 1991). Crop losses and control costs in Michigan due to Colorado potato beetle may be as high as $8 to $14 million per year in years when insecticide resistance problems are severe such as 1991-1994 (Grafius 1997). Georgiou and Lagunes-Tejeda (1991) reported that the Colorado potato beetle is resistant to more than 25 insecticides worldwide. It is believed that Colorado potato beetle is "pre-adapted" for insecticide resistance due to mechanisms it uses to deal with the glycoalkaloid toxins of its host plants (*Solanum* species) (Weber and Ferro 1994). Colorado potato beetle was one of the first insects to show insecticide resistance to DDT (Forgash 1985). This occurred in 1952 and presently in Michigan and throughout most of its range, Colorado potato beetle has some level of resistance to almost all registered insecticides, thus increasing the cost of potato production (Grafius 1997).

Transgenic potatoes expressing *Bacillus thuringiensis* Cry 3A delta-endotoxins (*Bt*) represent an alternative control for Colorado potato beetle. However, adoption has been slow due to public concern over safety, cost of the transgenic varieties and limited choice of varieties. Also, Colorado potato beetle resistance to *Bt* is a realistic possibility (McGaughley and Whalon 1992). Clearly there is a need for a sustainable IPM program that will safely and economically manage Colorado potato beetle.

The Potential of Entomopathogenic Nematodes

Nematodes (Phylum Nemata) are bilaterally symmetrical unsegmented pseudocoelomates (Maggenti 1981). The nematode body is generally elongate, cylindrical, and covered by cuticle secreted by hypodermal cells; however, many forms can exist, especially in the parasitic types (Maggenti 1981). Nematodes exist in almost every habitat on earth including marine, freshwater, and terrestrial and, in numbers, they exceed all other metazoa. In these habitats, nematodes are most numerous as the free-living type, but are most studied and understood in associations with plants and animals (Maggenti 1981). Associations existing between nematodes and invertebrates have recently become widely studied for their potential as biological control agents. These associations with invertebrates include phoresis, parasitism, and pathogenesis (Poinar 1975).

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae (Maggenti 1981) are obligate pathogens of insects. These families occur in the Order Rhabditida and their potential for use as biological control agents is due to important characteristics. First, they are the only nematodes that carry mutualistic

bacteria used to infect an insect host. Also, entomopathogenic nematodes have a wide host range that includes most insect orders and the nematodes can be mass-produced at a small cost (Poinar 1990). Finally, the nematodes usually kill their hosts within 48 h (Kaya and Koppenhöfer 1999).

Within Steinernematidae, there is one described species in the genus *Neosteinernema* and 24 species of *Steinernema* (Travassos). Heterorhabditidae contains one genus, *Heterorhabditis* (Poinar), with 7 described species (Kaya and Koppenhöfer 1999). New species are discovered each year. Entomopathogenic nematodes have been isolated from every continent except Antarctica (Kaya and Koppenhöfer 1999). The worldwide distribution of entomopathogenic nematodes suggests that many more species will be available for discovery, research, and biological control.

History of Entomopathogenic Nematodes for Control of *Leptinotarsa decemlineata*

Efforts to control Colorado potato beetle with entomopathogenic nematodes started four decades ago (Welch and Briand 1961). Depending on the nematode, however, results range from variable effects (Welch and Briand 1961, MacVean et al. 1982, Toba et al. 1983, Wright et al. 1987, Cantelo and Nickle 1992, Nickle et al. 1994, Berry et al. 1997) to promising potential for Colorado potato beetle control (Berry et al. 1997, Cantelo and Nickle 1992, Choo et al. 1989). Although the studies are limited and the experimental models vary greatly, the reasons for the different responses seem to be variations in nematode pathogenicity (Berry et al. 1997, Cantelo and Nickle 1992, Wright et al. 1987), variations in the susceptibility of Colorado potato beetle instars, whether nematodes were applied to the foliage (MacVean et al. 1982) or soil (Wright et al. 1987,

Cantelo and Nickle 1992, Berry et al. 1997), and edaphic factors (Koppenhöfer et al. 1995, Kung et al. 1990). I hypothesize that entomopathogenic nematodes can provide effective control of Colorado potato beetle when edaphic factors, timing, and application site are favorable.

Heterorhabditis marelatus

Heterorhabditis marelatus Liu and Berry is a recently discovered entomopathogenic nematode in the family Heterorhabditidae. *H. marelatus* seems to be highly pathogenic to Colorado potato beetle (Berry et al. 1997). *H. marelatus* should prove useful in management of Colorado potato beetle in Michigan for the following two reasons: 1) it was originally found in Seaside, Oregon and is adapted to cool temperatures common in potato production regions (Liu and Berry 1996); 2) like other *Heterorhabditis* spp., it actively searches for a suitable host within the soil (Poinar 1979).

The life cycle of *H. marelatus* is similar to other *Heterorhabditis* species (Fig. 1.2) (Liu and Berry 1996, Poinar 1990). The third stage infective juvenile is the only free-living stage of the nematode. The infective juvenile is adapted for locating a host and for survival within the environment for long periods as follows: 1) the mouth and anus are closed; 2) it has large reserves of lipids and numerous mitochondria; 3) the walls of the intestine and pharynx are closed together; and 4) the infective juvenile retains the second stage cuticle. These adaptations serve to sustain the infective juvenile, to reduce desiccation, to prevent infection or predation of the infective juvenile and to allow high mobility. Infective juveniles actively search for an insect host and, upon discovery, enter the host through natural openings (e.g., mouth, anus or spiracles) or may be able to enter

through the cuticle by using two small subventral teeth. Once inside the host hemocoel, a mutualistic bacterium, *Photorhabdus*, is released and kills the host within 48 h by septicemia. This mutualistic bacterium is located in the ventricular portion of the nematode intestine. The bacteria cause the insect cadaver to change to an orange color and the cadaver glows in the dark 2 d after infection. After nematode infection *H. marelatus* juveniles develop into hermaphroditic females in 4 d; thus only one infective juvenile needs to enter the host for progeny production (Kaya and Stock 1997). The hermaphroditic females lay eggs in inverse proportion to nematode density inside the host, with high numbers being laid during low nematode density. The bisexual generation, with males and females, is produced from these eggs in about 2 d. The nematodes feed on the mutualistic bacteria and host tissues (Kaya and Stock 1997). Males and females mate and produce eggs that will either become infective juveniles or another bisexual generation. This depends on nematode density and nutrient availability; high nematode density or low nutrient availability cue the formation of infective juveniles (Popiel et al. 1989). An alternate form of the third stage nematode exists in bisexual males and females that do not exhibit the same "free-living" characteristics as the third stage infective juveniles. If adequate moisture is available, the infective juveniles will leave the host to search for another insect host.

Photorhabdus sp. **Bacterial Symbionts**

All nematodes in the families Steinernematidae and Heterorhabditidae are associated with a mutualistic bacterium in the genus *Photorhabdus* (Kaya and Stock 1997). It is primarily this bacterium that is pathogenic to the insect host; however, the

nematode is needed for insect mortality because of the complex association between the nematode and bacterium. Different species of bacteria may exist for each nematode (Fischer-Le Saux et al. 1999). According to Liu et at. (2001), the *Photorhabdus* species isolated from *H. marelatus* appears to be very distinct from other known *Photorhabdus* species, although it is closely related to *P. temperata.*

The advantages to *Photorhabdus* of this association are protection and transport. The bacterium cannot survive well in soil or water (Poinar 1979) and requires the infective juvenile for protection. Bacterial cells remain inside the intestine of the nematode until released inside the insect host. In this way, the nematode is also supplying transport to each insect host. Once inside the insect hemocoel, the nematode inhibits the host's antibacterial defense, thus providing another form of protection to *Photorhabdus* (Kaya and Koppenhöfer 1999). In turn, the bacteria provide nutrients (Akhurst and Boemare 1990) and antibiotics (Akhurst 1981) for the nematodes. Nutrients are in two forms: 1) the bacteria transform host tissues into a food source and 2) the bacteria are a food source for the nematode (Kaya and Koppenhöfer 1999). The nematodes are unable to reproduce without nutrients provided by the bacteria (Akhurst and Boemare 1990). Antibiotics produced by the bacteria inhibit a wide range of other bacteria, yeast, and fungi (Kaya and Koppenhöfer 1999, Akhurst 1981). The antibiotics that have been isolated from *Photorhabdus* are hydroxystilbenes and anthroquinones (Kaya and Koppenhöfer 1999).

Efficacy Issues

A number of factors play important roles in the survival, pathogenicity, and mobility of entomopathogenic nematodes: 1) ultraviolet light causes high mortality and a great decrease in pathogenicity within minutes (Gaugler et al. 1992); 2) desiccation and high nematode mortality occur with foliar applications of nematodes (Bélair et al. 1998); 3) soil moisture is required for nematode movement (Kondo and Ishibashi 1985), persistence, and infectivity (Koppenhöfer et al. 1995); 4) soil type greatly affects nematode survival (Smith 1999) and mobility, with sandy soils being the most favorable; and 5) temperature plays a significant role in pathogenicity of entomopathogenic nematodes (Smith 1999, Saunders and Webster 1999), but optimal temperatures vary by species.

The effects of ultraviolet light and dessication suggest the need for soil rather than foliar application of nematodes for the control of Colorado potato beetle. Since only mature larvae, pupae, and over-wintering adult Colorado potato beetle occur in the soil, timing of application may be a critical factor for effective control. Although prior work addressed the effects on other heterorhabditids of soil moisture, texture, and temperature individually (Koppenhöfer et al. 1995, Kung et al. 1990), further studies need to address how all these factors interact to affect the development of *H. marelatus*.

Significance of *Heterorhabditis marelatus*

Chemical control of Colorado potato beetle has led to high levels of resistance to most available insecticides. Entomopathogenic nematodes offer an attractive alternative to chemical insecticides for control of Colorado potato beetle. Although

entomopathogenic nematodes have great potential as bio-pesticides, they have not been used to control Colorado potato beetle on a large scale because of their inability to survive in an exposed environment. Since 90% of the potatoes in Michigan are irrigated, this may provide a more suitable environment for the survival of the nematodes in the soil. In the past, the high cost of nematode production did not allow for economically feasible control of Colorado potato beetle (Welch and Briand 1961, MacVean et al. 1982, Toba et al. 1983, Wright et al. 1987). However, advances in nematode production quality and quantity will undoubtedly reduce costs (Connick et al 1993, Georgis 1990).

Soil type and application timing may be critical factors in effective Colorado potato beetle control, but current research has not focused on these factors. There has been no study on how *H. marelatus* develops in sandy loam and muck soil types that are suitable for potato production. Also, application must be made when Colorado potato beetle is most susceptible to infection. The highest susceptibility may occur during the Colorado potato beetle stage when nematodes are the most pathogenic and when nematodes and Colorado potato beetle coincide in the same environment.

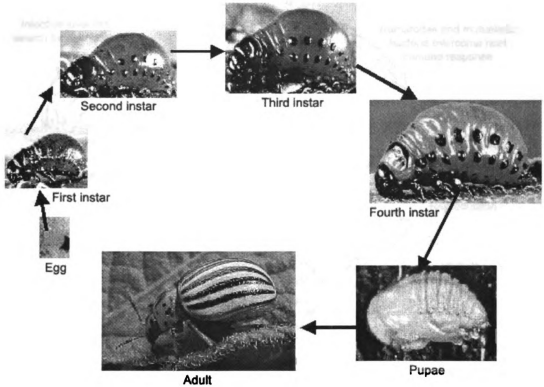

Figure 1.1. *Leptinotarsa decemlineata* lifecycle. After successful oviposition, eggs hatch in 4-8 days Colorado potato beetle has four larval instars and at maturation, the fourth instar drops to the ground to pupate beneath the soil. Adults emerge and begin to feed on potato foliage, mate and lay eggs.

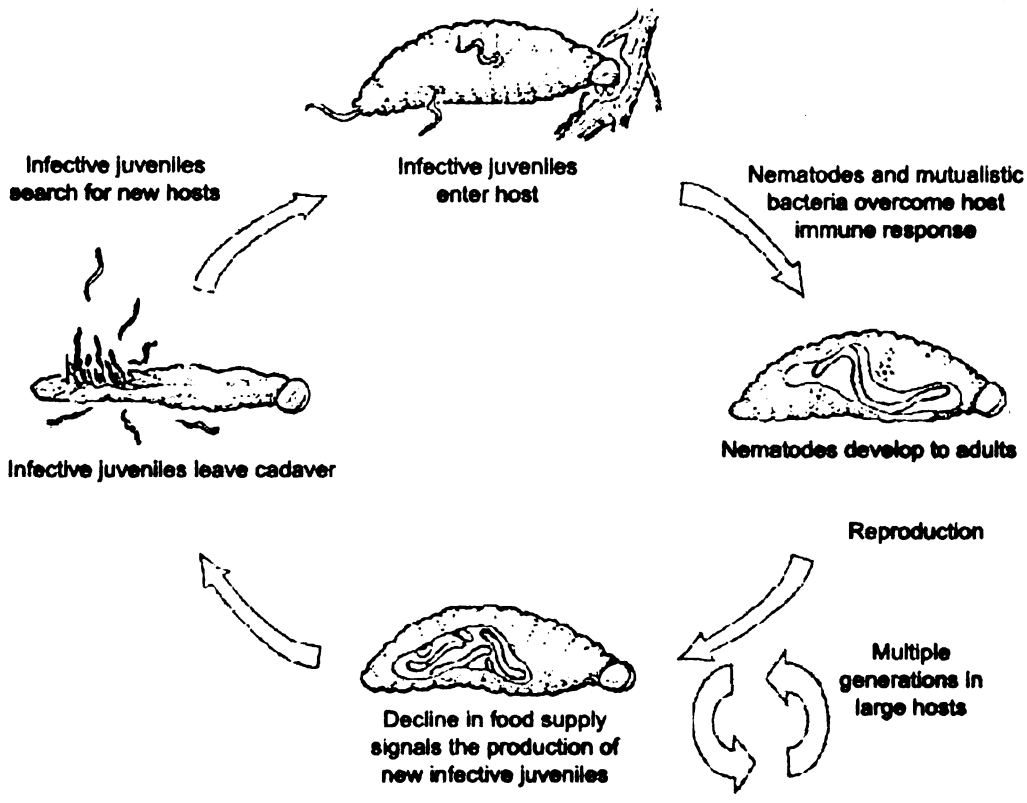

Figure 1.2. The life cycle of *Heterorhabditis marelatus* is similar to other species within the genus *Heterorhabditis*. The third stage infective juvenile is the only free-living stage of the nematode. The infective juvenile enters a host and overcomes the host immune response with the help of the bacterial symbiont. Multiple generations occur (depending on host size) and infective juveniles leave the host cadaver to search for a new host (From Polavarapu 1999).

CHAPTER 2

GENERAL MATERIALS AND METHODS

***Galleria mellonella* rearing.** Wax moth larvae, *Galleria mellonella* L. (Pyralidae, Lepidoptera), were maintained using a modification of the technique described by Dutky et al. (1962). *G. mellonella* were obtained from Carolina Biological Supply Company, Burlington, NC as mature larvae which were placed in a rearing chamber at 30 °C with no light and allowed to pupate. Approximately fifty *G. mellonella* pupae were transferred to a 3.79 L plastic container with tightly folded wax paper as an oviposition site. Eggs were collected from the wax paper and transferred to an artificial diet (80 g bran, 60 g wheat flour, 60 g cornmeal, 20 g brewer's yeast, 100 ml glycerin and 50 ml water). Dry ingredients were mixed first and then glycerin and water were added. This media was placed in 0.5 L Mason jars leaving 1/3 of the top of the jar empty. Then approximately 150 *G. mellonella* eggs were placed in each jar and mixed in the media. Lids for the jars were two pieces with an outer ring and inner circle. Wire screen (1 mm mesh) and 70 mm diam. Whatman no. 2 filter paper was used as a lid, held in place by the outer screw-on Mason jar ring. (Fig. 2.1). The filter paper was used to prevent contamination and escape of first and second instars. The wire screen was used to prevent larger larvae from escaping (third and fourth instars) from the jar. After media and eggs were placed in jars, the jars were kept at 30 °C with no light. Larvae migrate from the artificial diet to the empty space left at the top of the jars when it is ready to pupate. At this point, the fourth instar *G. mellonella* were collected from the jars to be

inoculated with *H. marelatus*. Larvae were collected and maintained as needed for nematode cultures and persistence studies.

In the spring of 2000, the USDA APHIS PPQ Invasive Pests Management Laboratory in Niles, Michigan, as part of a project entitled: The Niles Lab Partnership: Advancing Biological Control for Michigan's Plant Industries, established a colony of *G. mellonella*. The USDA Niles Center supplied *G. mellonella* throughout the 2000 field season and stock cultures were maintained at Michigan State University throughout the year.

Nematode Rearing. *G. mellonella* were used as a host for entomopathogenic nematode production because rearing costs are low and maintenance of the culture is easy. *H. marelatus* strain OH10 was obtained under a contractual agreement from Oregon State University, Corvallis, OR and maintained on last instar *G. mellonella* using a modification of the method described by Dutky et al. (1964). Whatman No.2 filter paper (90 mm diam.) was placed in the lid of an inverted 100 x 15 mm petri dish and 200 *H. marelatus* infective juveniles were applied to the filter paper in 1 ml of water. Ten *G. mellonella* larvae were placed on the Petri dish lid and covered with the inverted bottom for (Fig. 2.2). *G. mellonella* were then placed in a plastic bag to prevent desiccation and were kept in a rearing chamber at 20 °C with no light for 7 d.

Nematodes were extracted using a modification of the technique described by White (1927). One week after infection, *G. mellonella* cadavers were placed in a modified White trap (Fig 2.3) (a 60 mm diameter inverted Petri dish cover placed in a 150 mm diameter, 20 mm deep Petri dish). A piece of moistened 55 mm Whatman no. 2 filter paper was placed into the 60 mm inverted Petri dish cover and *G. mellonella*

cadavers were added to the moistened filter paper. The 60 mm Petri dish cover with infected cadavers was placed inside a 150 mm Petri dish filled half way with sterile distilled water. *H. marelatus* was collected from the modified White trap by removing the inner Petri dish and pouring the water with infective juveniles into a 4 L flask. More sterile distilled water was added to the outer dish so that *H. marelatus* harvest could continue daily for approximately 1 wk. Infective juvenile (third stage) nematodes were allowed to settle to the bottom of the collection flask for 30 min. The water was decanted and new water was added to the flask. This was done three times to eliminate contaminates, such as abiotic debris and bacterial infection. One drop of a 2 % solution of sodium bicarbonate and one drop of Triton X-100 was added to the nematode solution. Sodium bicarbonate prevents nematode clumping and Triton X-100 was added to prevent the infective juveniles from sticking to the surface of the flask. Infective juvenile nematodes were stored at 4 °C in distilled water aerated by an aquarium pump (Kaya and Stock 1997). Lab assays indicated that high survival occurred for up to 30 d. Nematodes for use in field experiments were stored for a maximum of 7 d and inspected microscopically for health and vigor before use.

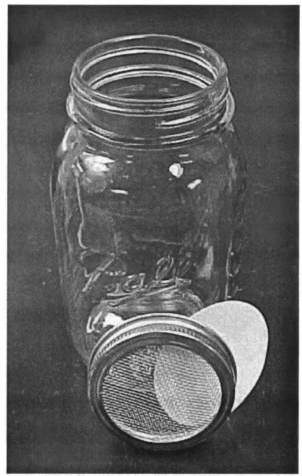

Figure 2.1. 0.5 L Mason jar used for rearing *Galleria mellonella*. Aluminum screen (1 mm openings) and 70 mm diameter Whatman no. 2 filter paper was placed in the outer ring of the Mason jar. The filter paper was used to prevent contamination and keep first and second instars from escaping from the jar. The wire screen was used to prevent larger larvae from escaping (third and fourth instars) from the jar.

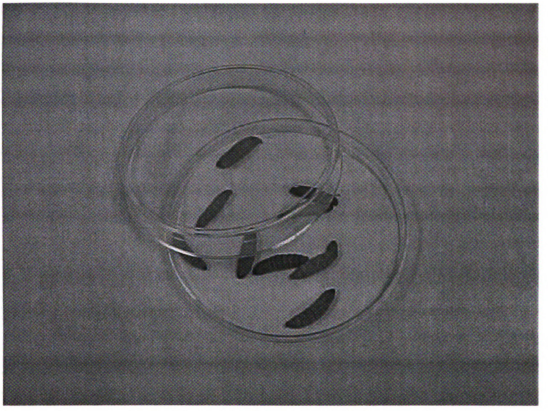

Figure. 2.2. The technique used to infect *G. mellonella*. Whatman No.2 filter paper (90 mm diameter) was placed in the lid of a 100 x 15 mm petri dish and 200 *H. marelatus* infective juveniles were applied to the filter paper in 1 ml of water. Ten *G. mellonella* larvae were then placed into the Petri dish and the lid was covered with the inverted Petri dish bottom.

Figure. 2.3. A modified White trap. A 60 mm diameter inverted Petri dish cover was placed in a 150 x 20 mm Petri dish. A piece of moistened 55 mm Whatman no. 2 filter paper was placed into the 60 mm inverted Petri dish cover and *G. mellonella* cadavers were added to the moistened filter paper. The outer Petri dish (150 x 20 mm) was filled half way with sterile distilled water.

CHAPTER 3

EFFECTS OF SOIL MOISTURE AND SOIL TEXTURE ON THE SURVIVAL AND PATHOGENICITY OF *Heterorhabditis marelatus* (RHABDITIDA: HETERORHABDITIDAE)

INTRODUCTION

Crop losses and control costs in Michigan due to Colorado potato beetle may be as high as $8 to $14 million per year in years when insecticide resistance problems are severe such as 1991-1994 (Grafius 1997). Insecticides are the most commonly used means to control Colorado potato beetle, but there is an increasing problem of insecticide resistance (Grafius 1997, Georgiou and Lagunes-Tejeda 1991). Georgiou and Lagunes-Tejeda (1991) reported that the Colorado potato beetle is resistant to more than 25 insecticides worldwide. It is believed that Colorado potato beetle is "pre-adapted" for insecticide resistance due to mechanisms it uses to deal with the glycoalkaloid toxins of its host plants (*Solanum* species) (Weber and Ferro 1994). Colorado potato beetle was one of the first insects to show insecticide resistance to DDT (Forgash 1985). This occurred in 1952 and presently in Michigan and throughout most of its range, Colorado potato beetle has some level of resistance to almost all registered insecticides, thus increasing the cost of potato production (Grafius 1997). The destructive ability and high levels of resistance in Colorado potato beetle to most available insecticides, has led to the need for additional control tactics against this pest.

Entomopathogenic nematodes are among the biological control agents that provide a safe alternative to complete reliance on chemical insecticides. Although prior studies have addressed the efficacy of entomopathogenic nematodes for Colorado potato beetle control (Welch and Briand 1961, MacVean et al. 1982, Toba et al. 1983, Wright et al. 1987, Choo et al. 1989, Cantelo and Nickle 1992, Nickle et al. 1994, Berry et al. 1997), few have focused on the effects of soil edaphic factors as related to pathogenicity against Colorado potato beetle (Koppenhöfer et al. 1995). *H. marelatus* (Liu and Berry) has the potential of becoming part of current IPM programs for continued control of Colorado potato beetle. However, there is a need to study the effect of soil edaphic factors on the pathogenicity and survival of *H. marelatus* before field scale studies were conducted.

As soil dwelling organisms that parasitize insects across multiple habitats, entomopathogenic nematodes are exposed to many edaphic and environmental adversities. For example, entomopathogenic nematodes are sensitive to soil moisture (influenced by soil texture) and by solar radiation (Kondo and Ishibashi 1985, Gaugler et al. 1992, Koppenhöfer et al. 1995, Smits 1996, Kaya and Stock 1997). Therefore, the optimal edaphic factors need to be determined for this species before an attempt is made to control Colorado potato beetle. Previous work on other entomopathogenic nematode species provides clues as to what should be considered. First, soil moisture is required for nematode movement (Kondo and Ishibashi 1985), persistence, and infectivity (Koppenhöfer et al. 1995). Also, soil type greatly affects nematode survival and mobility, with sandy soils being the most favorable (Smith 1999).

The objective of this study was to determine how (1) soil texture and (2) soil moisture affect the pathogenicity and survival of *H. marelatus*.

Materials and Methods

Nematode Rearing. Wax moth larvae, *Galleria mellonella* L., were used as a host for entomopathogenic nematode production because the rearing costs are low and maintenance of the culture is easy. *G. mellonella* were reared at 30 °C in rearing chambers as described by Dutky et al. (1962). The nematode was obtained from Oregon State University, Corvallis, OR and maintained on last instar *G. mellonella* using a modification of the method described by Dutky et al. (1964). Infected *G. mellonella* was kept in a rearing chamber at 20 °C. Nematodes were extracted using the technique described by White (1927) and stored at 4 °C in distilled water aerated by an aquarium pump.

Soil. The experiment included four soil types: loam, muck, sandy loam, and sand (Table 3.1). Muck and sandy loam soils were chosen because they are common in Michigan potato production. Sand and loam were chosen because of their high sand and silt content, respectively and represent the range of edaphic factors commonly inhabited by entomopathogenic nematodes. All soil was sterilized at 121 °C by autoclave and allowed to air dry for 1 wk before using 150 cc of each soil type in 500 cc plastic containers.

Moisture. Moisture levels were measured and adjusted using the filter paper technique (Fawcett and Collis-George 1967, Hamblin 1982, Kaya and Stock 1997) (Fig. 3.1) and included -400, -0.4, -0.02 and -0.001 MPa. These moisture levels represent a

wide range including dry soil (-400 MPa) and saturated soil (-0.001 MPa), all of which may be found under normal potato growing conditions.

Experimental Design. The experiment was a 4 x 3 factorial (48 containers; three replicates, four soil types, and four moisture levels). Nematodes were counted by taking six 20 µl drops of the nematode stock solution and counting the number of nematodes in each drop. The mean number of nematodes per 20 µl was multiplied by the total volume of the stock solution to estimate the total number of nematodes. This solution was then diluted to obtain a rate of 2,500 nematodes suspended in 1 ml of water. This amount was added to each container and moisture levels were re-measured. Water only was added to control containers. After inoculation of all 48 containers, the 500 cc plastic containers were placed in a growth chamber with no light at 20 °C. The experiment was conducted in the fall of 2001 and repeated in the spring of 2002.

Nematode Analysis. After 2 wks, infective juveniles were extracted from the soil in each container using a sieving technique (Kaya and Stock 1997). Sieves used were 18, 100, 200 and 635 mesh (1000, 150, 75 and 20 µm) stacked with 18 mesh on the top, 100, 200 and 635 mesh beneath. Nematodes were separated from the soil remaining in the 635 mesh sieve using a centrifugal flotation technique (Hooper and Evans 1993). After the extraction, surviving nematodes were separated from dead using a Baermann funnel (Kaya and Stock 1997) (Fig. 3.2) and counted. For each replicate, ten fourth instar *G. mellonella* larvae were then exposed to the surviving *H. marelatus* for 48 h. Dead *G. mellonella* were dissected to determine presence or absence of nematode infection. Pathogenicity was determined as percent *G. mellonella* mortality caused by nematodes.

Statistical Analysis. *H. marelatus* survival and persistence were analyzed by Friedman's two-way nonparametric ANOVA (Friedman 1937) using SAS general linear model (GLM) in conjunction with the rank procedure (SAS Institute 1988).

Linear regression analysis was used to analyze the effects of soil moisture on the survival of *H. marelatus* and the effects of soil moisture on the mortality of *G. mellonella* exposed to the surviving *H. marelatus* (SAS Institute 1988). Curvilinear regressions were used where mean *H. marelatus* survival or *G. mellonella* mortality was significantly higher at mid moisture levels than at low or high levels.

Results

Nematode Analysis. In the 2001 survival study nematode survival was not significantly different in any soil type ($P = 0.2077$, Table 3.2). In 2002 nematode survival was significantly affected by soil type ($P = 0.0001$). Overall survival of nematodes was highest in sandy loam followed by sand. Survival was lowest in loam and muck (Table 3.2).

Nematode survival was significantly increased by higher moisture in 2001 and 2002 ($P = 0.0003$, $P = 0.0001$, Table 3.2). Overall survival was lowest at -400 MPa in 2001 and 2002 (Table 3.2). Survival of nematodes was not significantly affected by the interactions of soil type and moisture level in 2001($P = 0.0594$, Table 3.2), but soil x moisture interactions were significant in 2002 ($P = 0.0019$, Table 3.2).

H. marelatus survival in loam increased as the soil moisture level increased in both the 2001 and 2002 studies ($r^2 = 0.75$ and 0.77, respectively; Fig. 3.3, 3.4). *H. marelatus* survival in muck also significantly increased at higher soil moisture in both studies ($r^2 = 0.73$ and 0.36, respectively). Nematode survival in muck increased as

moisture level increased until the highest moisture level of -0.001 MPa where survival dropped to near zero (Figs. 3.3 and 3.4).

H. marelatus survival in sandy loam was significantly increased by soil moisture in the 2001 and 2002 study (r^2 = 0.53 and 0.78, respectively). Nematode survival in sandy loam in 2001 increased from -400 to -0.4 MPa as moisture level increased, but survival in -0.02 and -0.001 MPa was lower than at -0.4 MPa (Fig. 3.3). In 2002, nematode survival in sandy loam increased as moisture level increased (Fig. 3.4).

H. marelatus survival in sand was significantly increased by soil moisture in the 2001 and 2002 study (r^2 = 0.89 and 0.92, respectively). Nematode survival in sand increased as moisture level increased (Figs. 3.3 and 3.4).

To measure pathogenicity of surviving *H. marelatus*, *G. mellonella* mortality was assessed. In 2001 and 2002 *G. mellonella* mortality was significantly affected by soil type (P = 0.0085, P = 0.0001, Table 3.3). Overall, mortality of *G. mellonella* was highest in sandy loam, followed by sand. Mortality was lowest in loam and muck (Table 3.3).

G. mellonella mortality was significantly increased by moisture (P = 0.0095, P = 0.0035, Table 3.3). Overall mortality was lowest at -0.001 and -400 MPa (Table 3.3). Mortality of *G. mellonella* was not significantly affected by the interactions of soil type and moisture level (P = 0.1261, P = 0.0755, Table 3.3).

In loam and muck, *G. mellonella* mortality was similar to *H. marelatus* survival. Also, in 2002 there was no *G. mellonella* mortality in loam, because no *H. marelatus* infective juveniles were recovered (Figs. 3.5 and 3.6).

G. mellonella mortality in sandy loam and sand was also very similar to *H. marelatus* survival. In sand, there was no significant difference in *G. mellonella*

mortality due to soil moisture level. However, in sandy loam, moisture levels between -0.4 and -0.001 MPa showed significantly higher mortality compared with dry soil (Figs. 3.5 and 3.6).

Discussion

Nematode Analysis. *H. marelatus* survival was highest in sandy loam and sand and lower in loam and muck. This is compatible with previous studies that found lower nematode survival with increasing amounts of clay and silt content (Hsiao and All 1996, Kung et al. 1990). Under our conditions studied, maximum survival of *H. marelatus* was obtained at soil moisture levels between -0.4 and -0.001 MPa. This is consistent with results of Koppenhöfer et al. (1995) but inconsistent with the results of Duncan et al. (1996) for *Steinernema riobravis*, who found higher nematode survival in dry soil possibly due to quiescence induced by low moisture availability (Kung et al. 1991, Duncan et al. 1996). One possible explanation for this difference is nematode species; *Steinernema riobravis*, *Steinernema carpocapsae* and *Steinernema glaseri* are better adapted to dry conditions (Koppenhöfer et al. 1995, Kung et al. 1991). These nematode species show a preference for the soil surface so it is essential to resist desiccation (Koppenhöfer et al. 1995). However, *Heterorhabditis* spp. do not prefer the soil surface and require higher moisture levels.

In 2001 regression analysis was done with a polynomial equation except in the survival study in loam and both studies in sand. A polynomial equation was used because there seemed to be a trend of decreasing *H. marelatus* survival and *G. mellonella* mortality at the highest moisture level of -0.001 MPa, possibly due to anaerobic conditions at these high levels of moisture. The exception to this was in sand, where *H. marelatus* survival and *G. mellonella* mortality both increased with high moisture levels. High variation was observed in sandy loam and sand at the highest moisture level (-0.001 MPa) in 2001 and 2002 (Figs.3.3, 3.4, 3.5, 3.6). This amount of variation was not found

in other soil types, perhaps because survival of nematodes consistently decreased at -0.001 MPa. The water saturated soil likely caused hypoxia of nematodes in loam and muck. It is possible that in sandy loam and sand, nematodes near the surface survived but those further in the soil died due to hypoxia. In 2002 lower *H. marelatus* survival and *G. mellonella* mortality at -0.001 MPa was also observed but on a much smaller scale. In the 2001 experiment, nematode survival was not significantly affected by soil type (P = 0.2077, Table 3.2) but in 2002 a significant effect was observed (P = 0.0001, Table 3.2). This difference may be due to the higher variation in 2001.

The results suggest that a heavier soil such as loam has a negative effect on *H. marelatus* survival. This negative effect is probably related to infective juvenile survival strategies. Infective juveniles do not feed, but must survive on lipid reserves (Liu and Berry 1996, Poinar 1990). The difficulty in moving through heavy soils may deplete these lipid reserves through increased rate of metabolism. Also, infective juvenile host finding may be better in sandy soil compared to loam due to slower, more difficult locomotion in loam (Smith 1999). The results suggest that many considerations must be made before applying *H. marelatus* for Colorado potato beetle control including water availability and soil type.

Overall, soil type and moisture seem to have a direct effect on nematode survival and an indirect effect on nematode pathogenicity. It is likely that an interaction affect between soil type and moisture level exists. A significant affect on nematode survival due to interactions between soil type and moisture level was only observed in the 2002 study (P = 0.0019, Table 3.2), however, in the 2001 study the P value was very close to significant at the 95% confidence level (P = 0.0594, Table 3.2). It is possible that with

enough moisture, any soil type can sustain *H. marelatus*; however, at high moisture levels in muck and loam soil, conditions may be anaerobic.

About 90% of Michigan potato growers irrigate their fields, which should provide an excellent environment for the survival and longevity of *H. marelatus* in the soil. However, for *H. marelatus* to be a successful biological control agent for Colorado potato beetle, irrigation should be managed differently when nematodes have been applied. Irrigation must be used for several weeks after application to ensure that soil remains moist enough for nematode survival.

The interaction between soil type and moisture content should be considered for effective management of Colorado potato beetle. *H. marelatus* survival was low in heavy soil, but may be optimized in cropping systems via natural rainfall and/or irrigation. Soil type must also be considered before *H. marelatus* is used for Colorado potato beetle control. *H. marelatus* may be less effective in heavy soil due to lower survival and pathogenicity compared to sandy soil. There may be a need for higher application rates of *H. marelatus* when used in loam. Under favorable conditions, *H. marelatus* may offer an additional tactic in the management of Colorado potato beetle.

Table 3.1. Content (% sand, silt and clay) for each soil type used in the study.

	% Sand	% Silt	% Clay
Loam	49.84	31.08	19.08
Muck	67.84	17.44	14.72
Sandy Loam	79.84	9.44	10.72
Sand	92.52	2.09	5.39

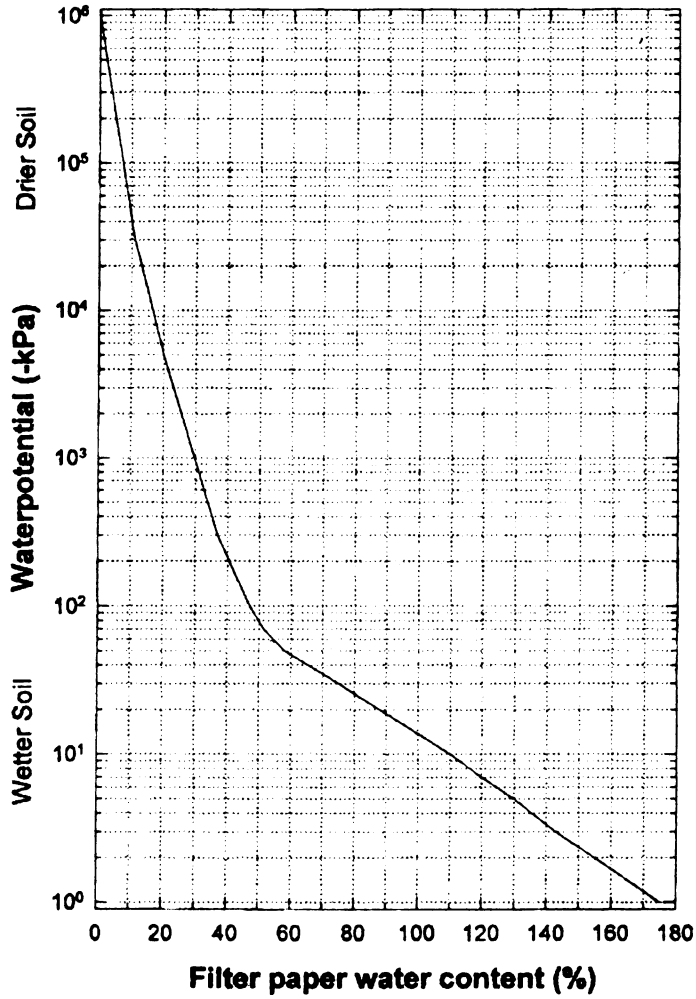

Figure 3.1. Calibration curve for Whatman no. 42 filter papers showing water potential in kilopascals (kPa) of soil against filter paper water content (from Kaya and Stock 1997). The dry weight of filter paper was measured before placing filter paper in soil. Once the filter paper equilibrated to soil moisture, the filter paper was retrieved and the wet weight of filter paper was measured. Final calculation of % moisture of filter paper was measured by subtracting dry weight from wet weight and dividing by dry weight of the filter paper. The % moisture was used to determine water potential from the graph.

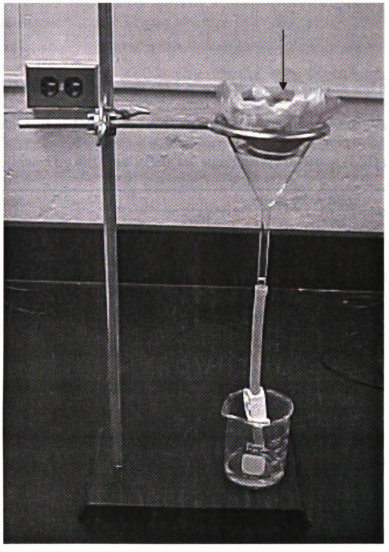

Figure 3.2. A Baermann funnel used in separating living nematodes from dead. Infective juveniles were placed in a moistened sponge (arrow) and wrapped in tissue and aluminum wire. The funnel was filled with sterile distilled water to the level of the sponge. Every 2-3 h, the clamp was released to collect living nematodes.

Table 3.2. Means and standard error (SE) of soil type, soil moisture and interaction effects on the survival of *Heterorhabditis marelatus* in 2001 and 2002. Values within each column followed by different letters are significantly different (LSD alpha = 0.05).

Factor	Suvival (% ± SE)	
	2001	2002
Soil Type	$P = 0.2077$	$P = 0.0001$
Loam	(4.58 ± 1.61) a	(2.0 ± 1.03) c
Muck	(6.17 ± 2.44) a	(3.67 ± 1.63) c
Sandy Loam	(22.33 ± 8.04) a	(34.75 ± 6.94) a
Sand	(9.0 ± 3.17) a	(19.67 ± 5.06) b
Moisture Level (MPa)	$P = 0.0003$	$P = 0.0001$
-400	(0.92 ± 0.61) B	(0 ± 0) B
-0.4	(17.25 ± 5.62) A	(18.75 ± 6.22) A
-0.02	(9.75 ± 2.41) A	(18.75 ± 4.61) A
-0.001	(14.17 ± 7.03) A	(22.58 ± 7.34) A
Soil/Moisture Interaction	$P = 0.0594$	$P = 0.0019$

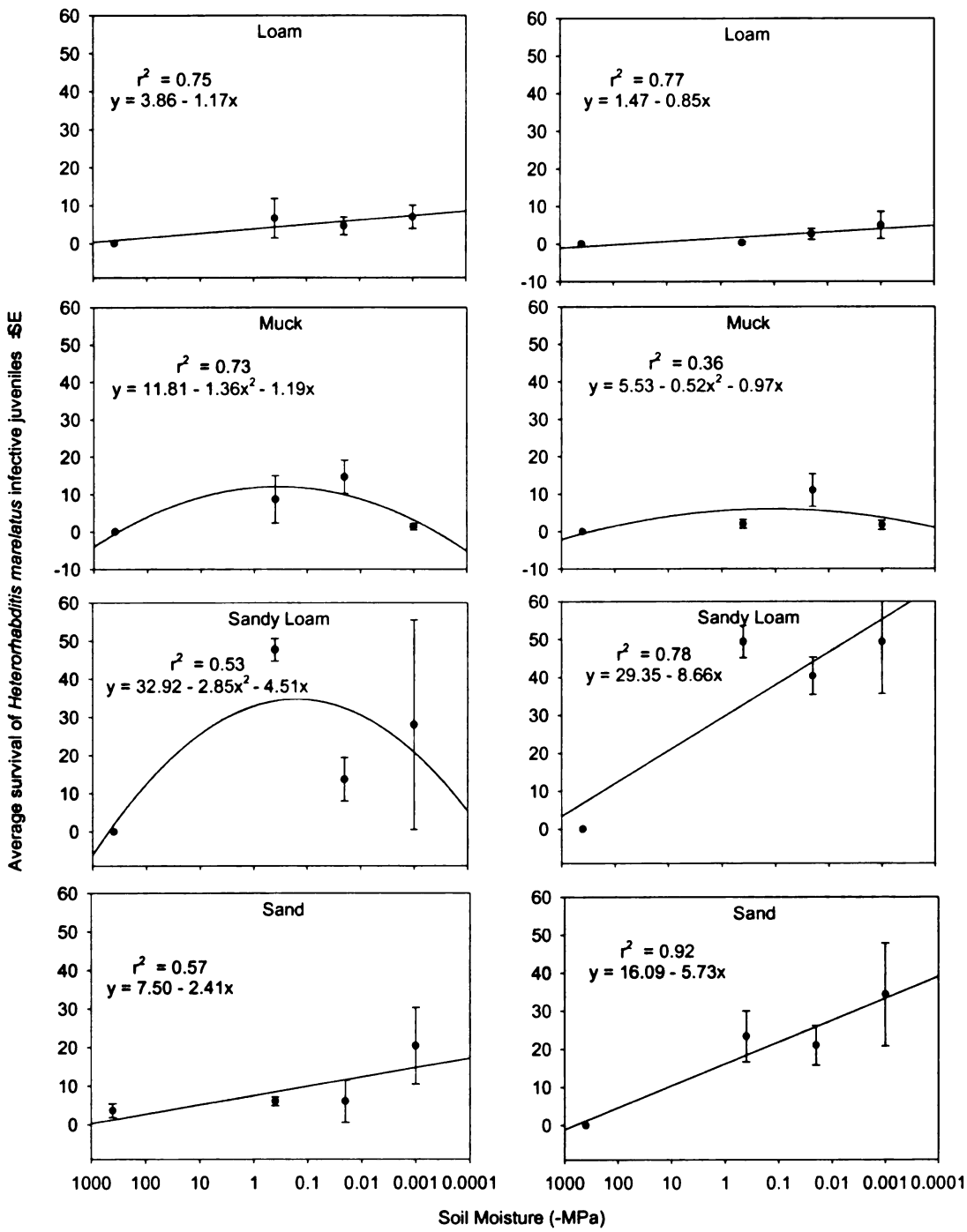

Figure 3.3. Average survival of *Heterorhabditis marelatus* infective juveniles within shown soil types and moisture levels in 2001 ±standard error.

Figure 3.4. Average survival of *Heterorhabditis marelatus* infective juveniles within shown soil types and moisture levels in 2002 ±standard error.

Table 3.3. Means and standard error (SE) of soil type, soil moisture and interaction effects on the mortality of *Galleria mellonella* in 2001 and 2002. Values within each column followed by different letters are significantly different (LSD alpha = 0.05).

Factor	Suvival (% ± SE)	
	2001	2002
Soil Type	$P = 0.0085$	$P = 0.0001$
Loam	(0.83 ± 0.83) b	(0 ± 0) c
Muck	(2.5 ± 1.31) b	(0.83 ± 0.83) c
Sandy Loam	(10.00 ± 3.02) a	(18.33 ± 4.41) a
Sand	(4.17 ± 1.93) ab	(7.50 ± 2.79) b
Moisture Level (MPa)	$P = 0.0095$	$P = 0.0035$
-400	(0 ± 0) B	(0 ± 0) B
-0.4	(7.5 ± 2.5) A	(7.50 ± 3.05) A
-0.02	(5.0 ± 1.51) A	(9.17 ± 3.58) A
-0.001	(5.0 ± 2.88) A	(10.0 ± 4.44) A
Soil/Moisture Interaction	$P = 0.1261$	$P = 0.0755$

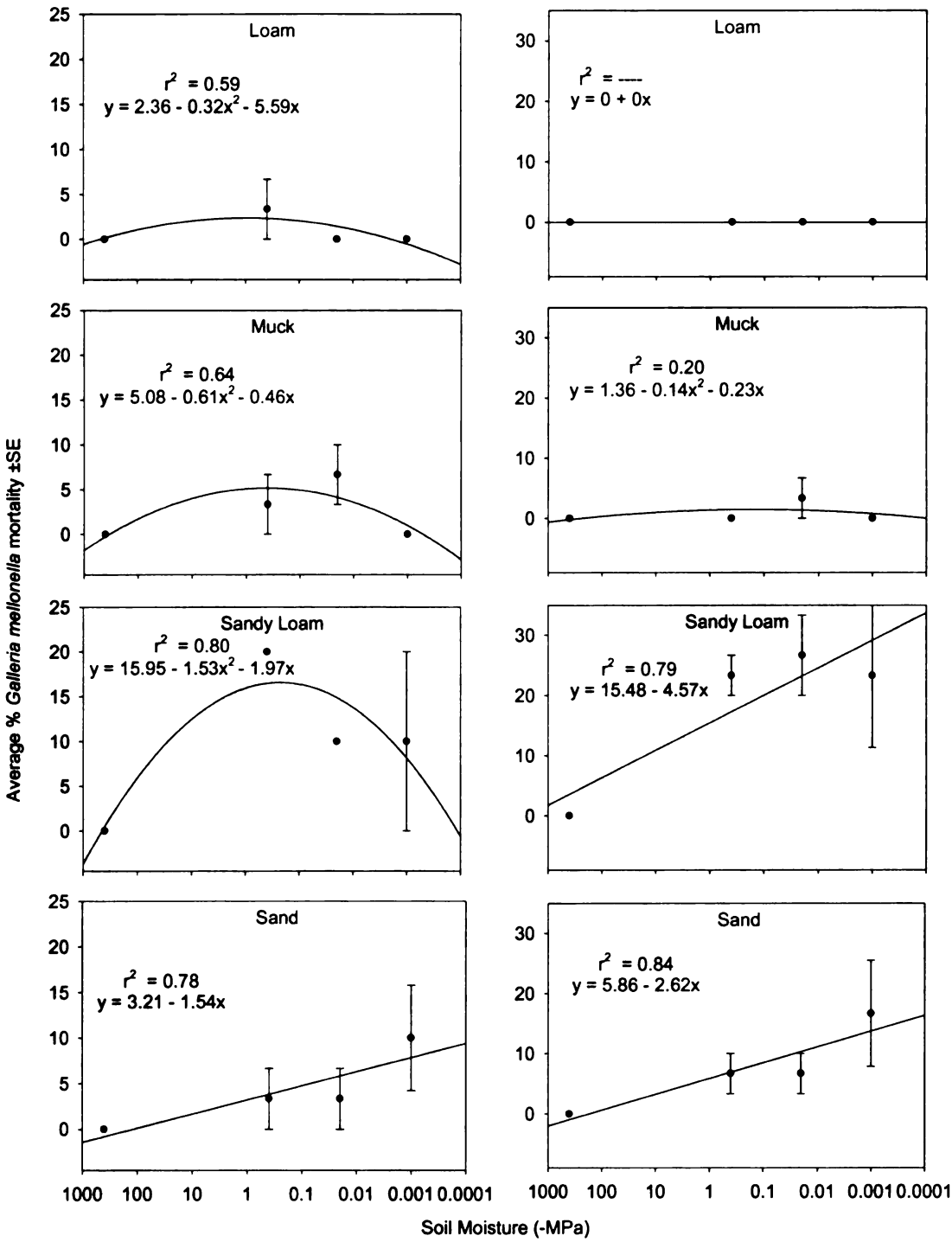

Figure 3.5. Average % mortality of *Galleria mellonella* exposed to extracted *Heterorhabditis marelatus* infective juveniles in 2001 ±standard error.

Figure 3.6. Average % mortality of *Galleria mellonella* exposed to extracted *Heterorhabditis marelatus* infective juveniles in 2002 ±standard error.

CHAPTER 4

EFFECT OF VARYING RATES OF THE ENTOMOPATHOGENIC NEMATODE *Heterorhabditis marelatus* (RHABDITIDA: HETERORHABDITIDAE) ON POPULATION DYNAMICS OF THE COLORADO POTATO BEETLE (COLEOPTERA: CHRYSOMELIDAE)

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are one of the most important vegetable crops in the United States. In Michigan, potatoes are valued at approximately $90-100 million/yr (Michigan Potato Industry Commission 1996). Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most destructive insect pest of potato, causing an estimated $13 million loss per year from 1990 to 1995, when insecticide resistance problems were severe (Grafius, 1997). Although insecticides provide most of the control of Colorado potato beetle in Michigan, some level of resistance to almost all registered insecticides has developed (Forgash 1981, Forgash 1985, Bishop and Grafius 1996, Grafius 1997, Georgiou and Lagunes-Tejeda 1991). Thus, increasing the cost of potato production.

The development of resistance to most available insecticides in Colorado potato beetle has led to the need for additional control tactics, including biological control. Efforts to control Colorado potato beetle with the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. have been tried with variable success. This, in part, appears to be limited by humidity (Campbell et al. 1985, Feng et al. 1994). Tipping et al. (1999)

showed that *Edovum puttleri* Grissell (Hymenoptera: Eulophidae) and *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) are not economically feasible on a large scale for control of Colorado potato beetle in tomatoes. Biological control using *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) has also been attempted. However, Colorado potato beetle eggs are not the optimal diet for *C. maculata* and they exhibit a preference for aphids, when available (Hazzard and Ferro 1991).

Entomopathogenic nematodes have shown promising potential for Colorado potato beetle control (Berry et al. 1997, Cantelo and Nickle 1992, Choo et al. 1989). A recently discovered species, *Heterorhabditis marelatus* (Liu and Berry), is highly pathogenic to Colorado potato beetle (Berry et al. 1997). It was originally found in Seaside, OR and is adapted to cool temperatures common in potato production regions (Liu and Berry 1996). While the habitat of its origin seems to indicate that *H. marelatus* may prove useful in the management of Colorado potato beetle, no information is available on its survival and pathogenicity under Michigan conditions. The objective of this study was to determine the survival and pathogenicity of *H. marelatus* to Colorado potato beetle under field conditions.

Materials and Methods

Experimental Site Description. The field study was conducted at the Michigan State University Montcalm Potato Research Farm in Montcalm County, MI (4321.183 N, 8510.484 W) during 2000 and 2001. The research farm has McBride sandy loam soil and artificial irrigation. "Snowden" potatoes were planted and maintained using standard commercial potato production practices including fertilization, irrigation, fungicide and herbicide treatments; no insecticides were used against Colorado potato beetle.

Weather data was recorded daily using an on-site weather station maintained by the Michigan State University Agricultural Weather Office. Data collected included maximum and minimum air temperature, maximum and minimum soil temperature, precipitation, relative humidity and estimated potential evapotranspiration.

Experimental Design and Nematode Inoculation. Targets for control were the soil inhabiting stages of the Colorado potato beetle (fourth instars entering the soil for pupation, pre-pupae, pupae and new adults); all are easily infected by *H. marelatus* under laboratory conditions (unpubl. data). Treatments were 0 (control), 333 million, 667 million or 1 billion nematodes/hectare and arranged in a randomized complete block design, replicated four times. Potatoes were planted with 86 cm spacing between rows and 23 cm between plants (16 May 2000, 8 May 2001); each plot was 4 rows x 14 m in 2000 and 4 rows x 13 m in 2001.

Nematode application was timed to correspond with the occurrence of fourth instar Colorado potato beetles (15 June - 5 July). A concentrated solution with the required number of *H. marelatus* infective juveniles was diluted in 2 liters of water and applied to the soil using a Chapin single nozzle hand-held sprayer (Chapin Manufacturing, Inc., Batavia, New York) at 207 kPa on 22 June 2000 and 26 June 2001. The sprayer was agitated continually to ensure uniform nematode mixing. Control plots received water only. Immediately following nematode application, plots were irrigated (1-2 cm) using a sprinkler irrigation system. Latitude and longitude of the area that received nematode application are 4321.183 N, 8510.484 W.

Colorado Potato Beetle Population Analysis. Colorado potato beetle adults, larvae and egg masses on six randomly selected potato plants in the middle two rows of

each plot were counted weekly before and after nematode application (4 June – 9 Aug 2000). In 2001, after nematodes had been applied, two 1 m^2 field cages (three plants each) were placed in each plot to control migration of beetles between plots and these plants were sampled weekly from 19 June – 31 July. Only numbers of adult Colorado potato beetles that emerged after nematode application (summer adults) were used to determine what effect the nematode had on beetle survival in the soil.

***H. marelatus* Persistence.** During 2000 and 2001, the presence and pathogenicity of *H. marelatus* was assayed by burying five fourth instar *G. mellonella* wrapped in nylon bags approximately 6 cm below the soil (two per plot), beginning 1 mo after nematode application. Exactly 1 wk later, *G. mellonella* samples were collected and dissected to determine *H. marelatus* infection. This persistence test was conducted four times each year from August to September. The test was also conducted in the spring of 2001 (April – May) to test for *H. marelatus* survival from the previous season.

Plant Damage Assessment. Potato defoliation in 2000 was estimated visually on a linear scale from 0-10, (0 to 100% defoliation). Defoliation was estimated on six randomly chosen plants in the middle two rows of each plot three times after nematode application (2, 9, 16 Aug 2000), when defoliation was most severe. The evaluator did not know which treatments had been applied to each plot. This assessment was not done in 2001 because potato defoliation from first generation larvae was very high prior to nematode application and little foliage remained by the time summer adults emerged, when the impact of the nematodes would have been apparent.

Statistical Analysis. Colorado potato beetle numbers, *H. marelatus* persistence and potato defoliation data were non-parametric (Shapiro-Wilk test, $P < 0.0001$), so data were analyzed using Friedman's test (Friedman 1937, SAS Institute 1988).

Results

Beetle Population Analysis. There were no significant differences between treatments in larval numbers prior to nematode application in 2000 or 2001 ($P>0.05$, Table 4.2, Figs. 4.1 and 4.2). Summer adult Colorado potato beetle started to appear on 12 July 2000, peaking on 26 July 2000. On 26 July, 2 Aug and 9 Aug 2000 adult numbers in control plots were significantly higher than numbers in nematode treated plots ($P=0.0001$, Table 4.2). However, there were no significant differences between treatment rates. There were no significant differences in adult beetle numbers between treatments and control plots in 2001 ($P=0.1225$, Table 4.2, Fig. 4.2).

***H. marelatus* Persistence.** *H. marelatus* was detected on every sample date in 2000. On 9, 23 and 30 Aug 2000, *G. mellonella* infection in treated plots ranged from 50 – 100% (Fig. 4.3). There was no *G. mellonella* infection in control plots, significantly less than in all other treatments (LSD alpha = 0.05). On 8 Sept 2000 infection ranged from 10 – 30% (Fig. 4.3) and means in two of the three treatments were significantly higher than in the control (LSD alpha = 0.05). In the spring following the 2000 field season and the end of the 2001 field season (Aug – Sept), the same methods were carried out, but no *G. mellonella* mortality occurred as a result of *H. marelatus* infection or infection by any other entomopathogenic nematode.

Damage Assessment. Nematode treatment in 2000 resulted in significantly less late season defoliation (Fig. 4.4). On 2 Aug 2000, plants in all nematode treatments had

significantly less defoliation than plants in the control (LSD $p< 0.05$). On 9 Aug 2000, there were no significant differences in defoliation between treatments and the control (LSD, $P>0.05$). On 16 Aug 2000, defoliation in the 1 billion nematodes/hectare treatment was significantly lower than in the control (LSD, $P<0.05$).

Discussion

Beetle Populations. Nematodes were targeted against the mature larvae (fourth instars), as they move into the soil to pupate, and against pupae and emerging adults (Bélair et al. 1998, Gaugler et al. 1992). The expected result was a decrease in adults emerging after the nematode application. Nematode treatment was effective in reducing the number of summer adults in 2000, but not 2001. The success of the *H. marelatus* for control in 2000 is probably the result of optimal soil edaphic factors. The sandy loam soil had high moisture throughout most of the season due to a wet summer and moderate temperatures. This is consistent with results found by Koppenhöfer et al. (1995). In 2001, however, there was not effective control of Colorado potato beetle with *H. marelatus*. This is probably due to a very hot and dry summer (Table 4.1). In years with such conditions, other forms of control should be used with *H. marelatus* for control of Colorado potato beetle. Increased irrigation after nematode application would also be recommended.

***H. marelatus* Persistence.** According to reports in the literature, the nematode should persist for only 2 - 6 wks after application (Georgis 1992, Smits 1996). However, in 2000 *H. marelatus* remained in the soil for greater than 11 wks. Again, this may be a result of optimal soil, moisture and temperature conditions for the nematode. This hypothesis is supported by the 2001 findings, where no *G. mellonella* larvae were killed by nematodes, even just 2 wks after application of *H. marelatus*, apparently due to hot and dry conditions.

Damage Assessment. It was clear from the analysis and from visual observations that *H. marelatus* resulted in significant levels of control of Colorado potato beetle in

2000. Although defoliation reached 65-70% in treated plots, this is compared with 90% defoliation in the control; also, this defoliation difference was attained without the use of insecticides for Colorado potato beetle control. Highest defoliation actually occurred in treated plots due to migration of adult beetles into these after untreated plots had been completely defoliated. This was the reason field cages were introduced in 2001, although *H. marelatus* apparently did not survive in 2001.

Results of this study indicate that the application rate of nematodes is perhaps much less important than environmental conditions. Application rates of *H. marelatus* could probably be decreased significantly below rates used in this research and commonly recommended for other entomopathogenic nematodes (Shields et al. 1999, Jouney and Ostlie 2000). This could make the use of these nematodes more economical for large scale commercial use that has previously been discouraged by high costs (Welch and Briand 1961, MacVean et al. 1982, Toba et al. 1983, Wright et al. 1987, Cantelo and Nickle 1992, Nickle et al. 1994, Jackson 1996, Berry et al. 1997, Jouney and Ostlie 2000).

For optimal control of Colorado potato beetle, *H. marelatus* should be used with other forms of control such as crop rotation, resistant varieties of potatoes and pesticides, where necessary. Mortality induced by *H. marelatus* may ultimately reduce insecticide use in Michigan potatoes, reduce selection pressure for insecticide resistance, and maintain the efficacy of existing insecticide products. *H. marelatus* has the potential of becoming part of current integrated pest management programs for continued control of Colorado potato beetle.

Table 4.1. Average rainfall, temperatures and Colorado potato beetle per plant

	2000	2001
Total Rain (mm) 1 June – 8 Aug	281.9	140.5
Average temperature (°C) 1 June – 8 Aug	19.2	20.8
Average number of Colorado potato beetles	3.9	16.6

Table 4.2. P Values, using Friedman's test (Friedman 1937), to compare Colorado potato beetle numbers in the four treatments (0, 333 million, 667 million or 1 billion nematodes/hectare) within each Colorado potato beetle life stage, showing no significant difference in egg and larval numbers (early season before nematode application). Summer adult numbers are after nematode application.

CPB Stage	P Value; Mean ± SE 2000	P Value; Mean ± SE 2001
Egg masses	0.9689 (0.91 ± 1.56)	0.6776 (1.10 ± 0.54)
First instar	0.0570 (2.31 ± 3.08)	0.8780 (2.44 ± 2.96)
Second instar	0.2994 (3.10 ± 2.87)	0.9267 (4.86 ± 3.78)
Third instar	0.3353 (2.47 ± 1.73)	0.2096 (12.13 ± 6.11)
Fourth instar	0.6254 (4.36 ± 2.59)	0.6640 (10.34 ± 4.97)
Summer adult	0.0001* (4.18 ± 1.84)	0.1225 (3.76 ± 3.06)

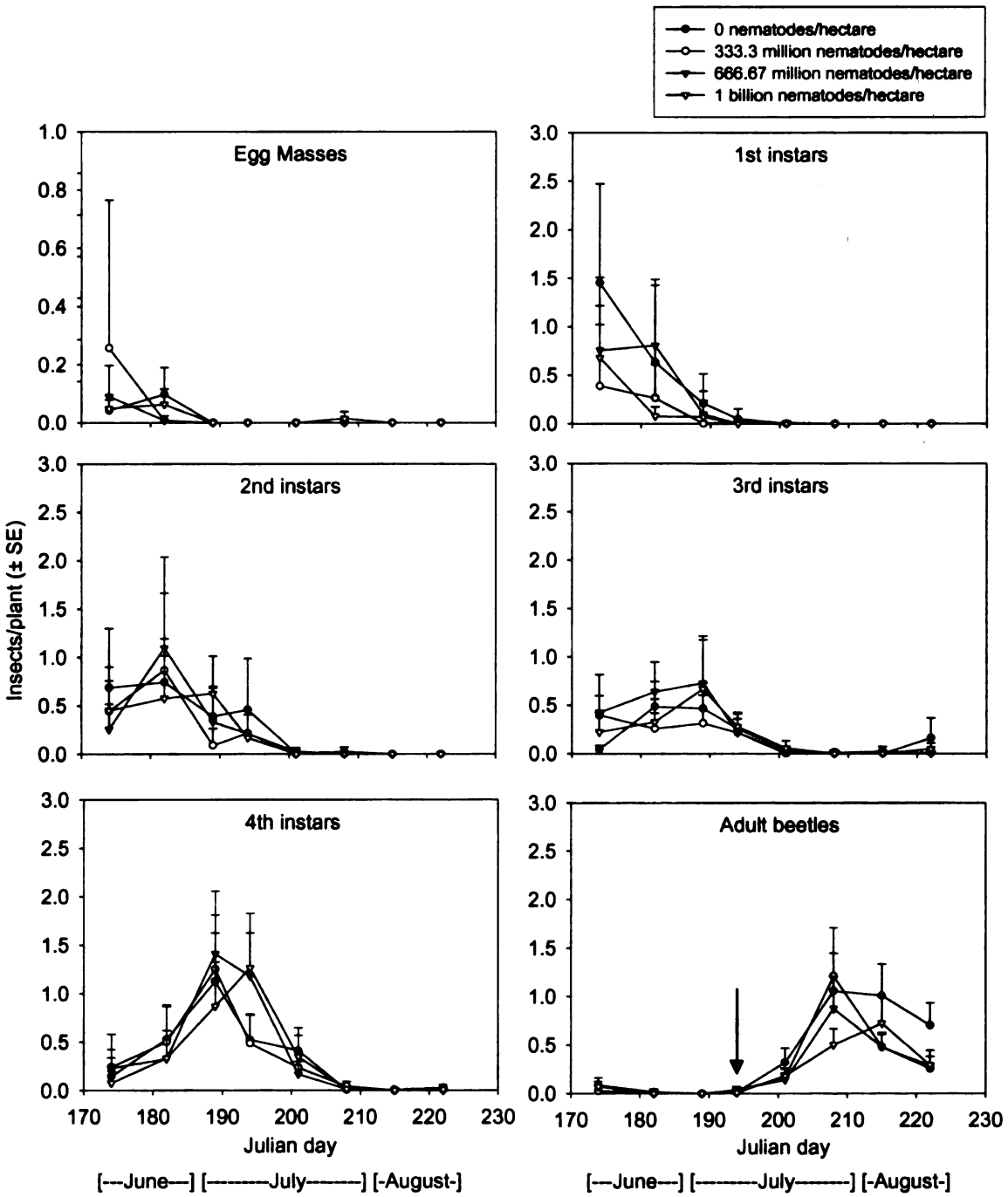

Figure 4.1. Mean numbers of Colorado potato beetle life stage per potato plant vs. Julian days in 2000 ± SE. The arrow in the "adults" graph indicates when the nematodes were applied to the soil.

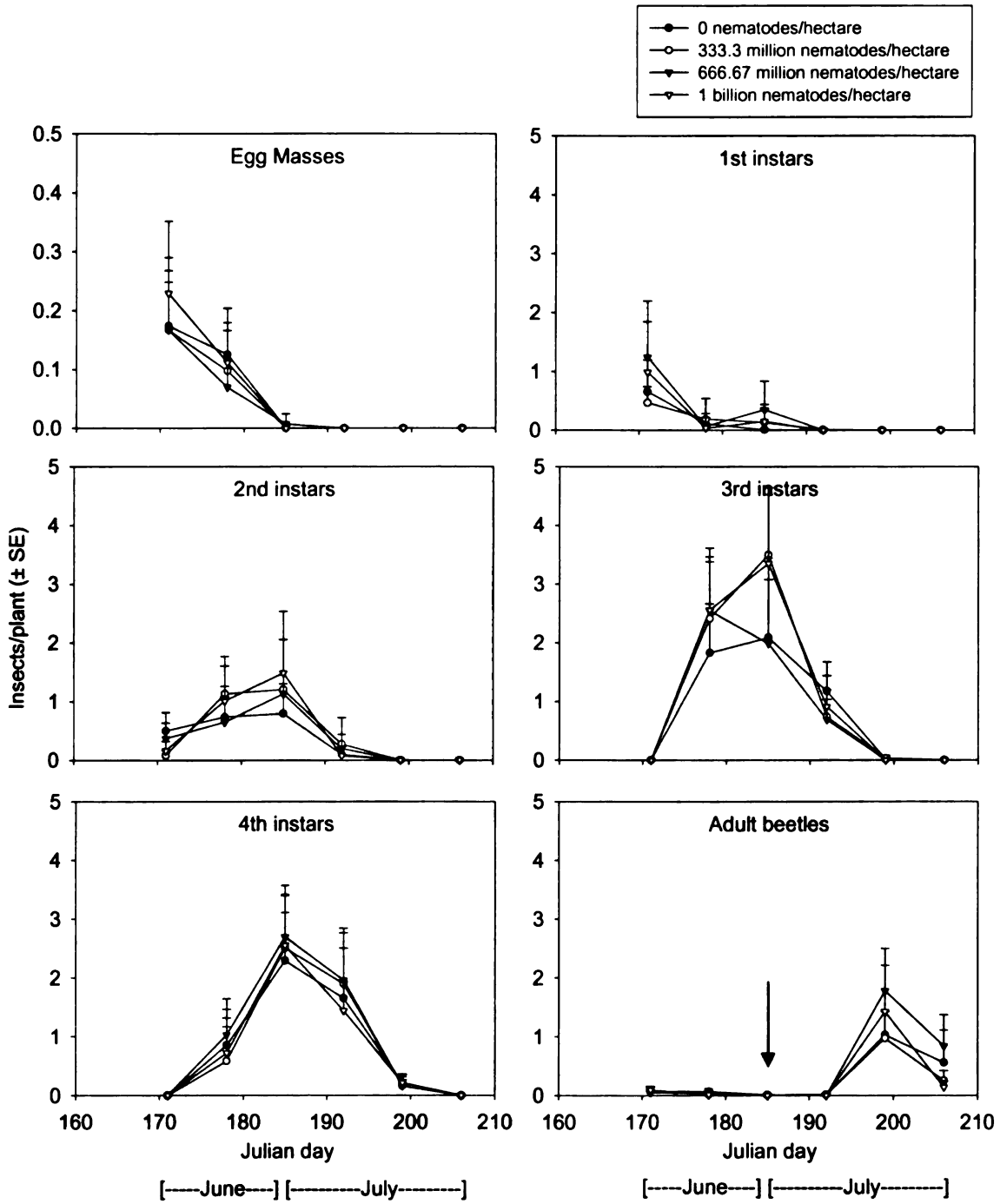

Figure 4.2. Mean numbers of Colorado potato beetle life stage per potato plant vs. Julian days in 2001 ± SE. The arrow in the "adults" graph indicates when the nematodes were applied to the soil.

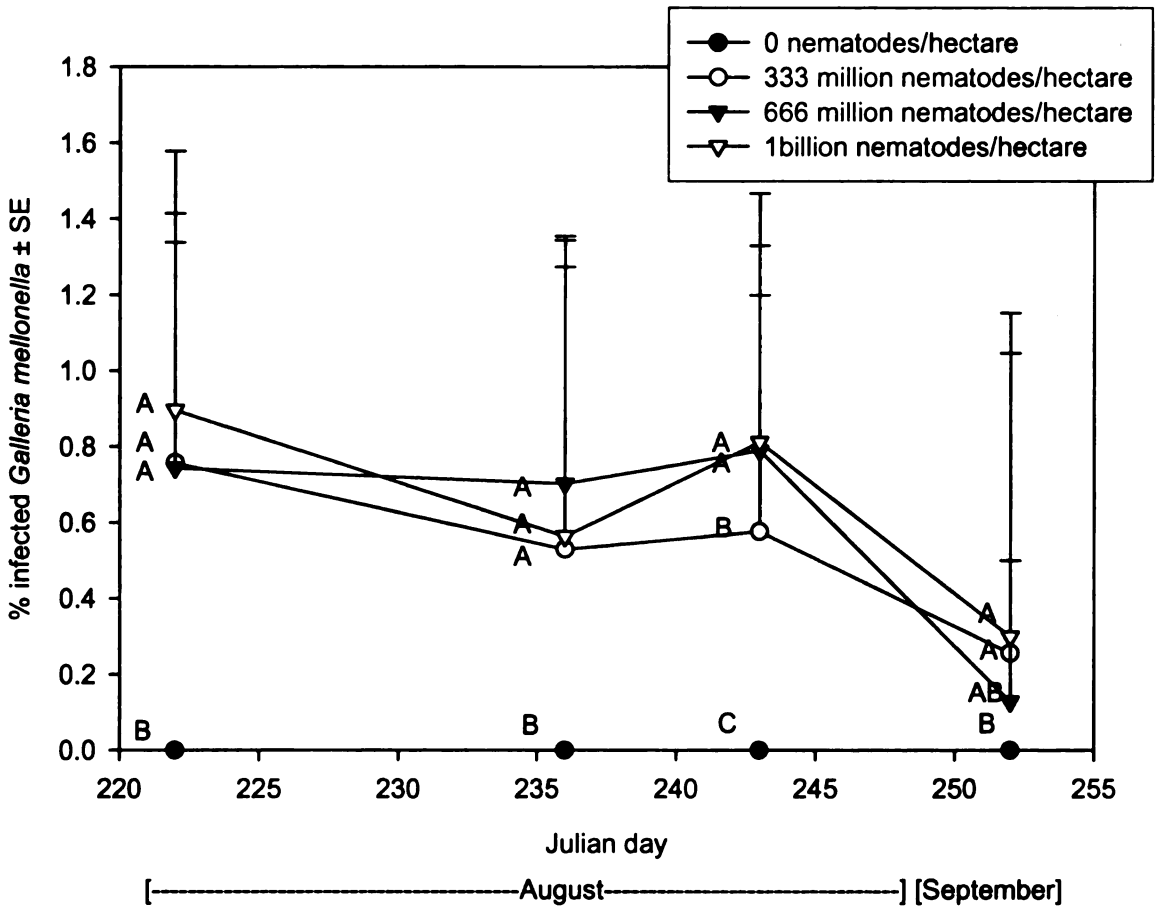

Figure 4.3. % infection of *Galleria mellonella* by *Heterorhabditis marelatus* vs. Julian days of *Heterorhabditis marelatus* in the field in 2000. Different letters (vertically) indicate significant difference between treatments (LSD alpha = 0.05).

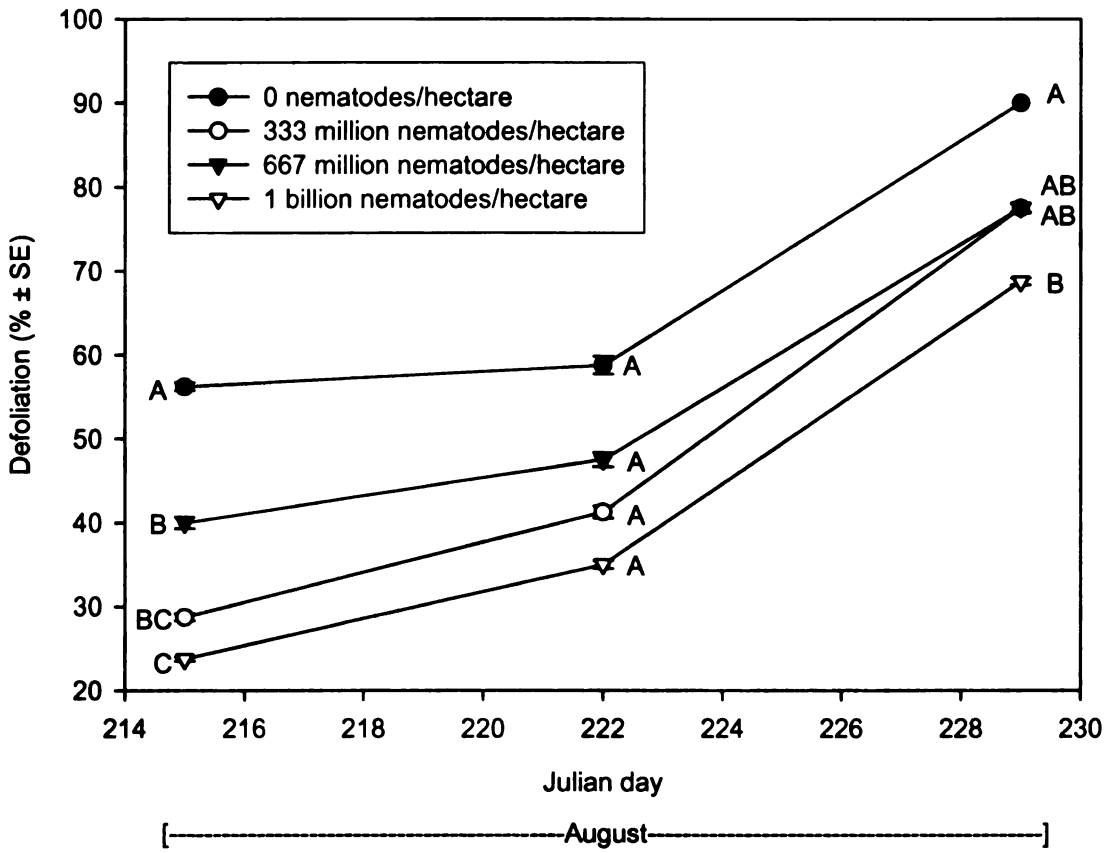

Figure 4.4. Percentage potato defoliation by Colorado potato beetle vs. *Heterorhabditis marelatus* rate in 2000. Different letters (vertically) indicate significant difference between treatments (LSD alpha = 0.05).

CHAPTER 5

EFFECT OF TIMING ON *Heterorhabditis marelatus* APPLICATION FOR COLORADO POTATO BEETLE (COLEOPTERA: CHRYSOMELIDAE) CONTROL

INTRODUCTION

Providing 15% of annual cash receipts, potato (*Solanum tuberosum* L.) is the most important vegetable crop in the United States (Anon. 1999) with a value of $2.9 billion in 2001 on 1.2 million acres. As far as crops grown for human food use in the United States, potatoes rank second behind wheat in importance. The billions of dollars generated from the chipping and food service industries further enhance revenues generated by potato growers (Anon. 1999). In Michigan, potatoes have a retail value of >$600 million (Michigan Potato Industry Commission 1996), approximately six times their farmgate value.

Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most destructive pest of potatoes throughout much of the Northeastern and Midwestern U. S. and Canada. Insecticides provide most of the control of Colorado potato beetle, however, some level of resistance to almost all registered insecticides has developed (Forgash 1981, Bishop and Grafius 1996, Grafius 1997, Ioannidis et al. 1991, Georgiou and Lagunes-Tejeda 1991). Transgenic potatoes expressing *Bacillus thuringiensis* Cry 3A delta-endotoxins (*Bt*), were available commercially beginning in 1995 but were not widely adopted because of public concern over safety, cost of the transgenic varieties and limited choice

of varieties available. Also, Colorado potato beetle resistance to *Bt* is a realistic possibility (McGaughley and Whalon 1992). Biological control does not provide significant control of Colorado potato beetle and, in fact, Harcourt (1971) determined that there were no natural mortality factors in potatoes untreated with insecticides that prevented Colorado potato beetle from overshooting its food supply. Clearly there is a need for a sustainable IPM program that will safely and economically manage Colorado potato beetle.

Heterorhabditis marelatus Liu and Berry is a recently discovered entomopathogenic nematode in the family Heterorhabditidae. *H. marelatus* seems to be highly pathogenic to Colorado potato beetle (Berry et al. 1997). *H. marelatus* should prove useful in management of Colorado potato beetle in Michigan for the following two reasons: 1) it was originally found in Seaside, OR and is adapted to cool temperatures common in potato production regions (Liu and Berry 1996); 2) like other *Heterorhabditis* spp., it probably actively searches for a suitable host within the soil (Poinar 1979).

The optimal rate of *H. marelatus* for Colorado potato beetle control was investigated by Cottrell (2002 Chapter 4). *H. marelatus* can effectively control Colorado potato beetle in the presence of optimal soil moisture and texture. Moist sandy soils are ideal for survival and pathogenicity of *H. marelatus* (Cottrell et al. 2002 Chapter 3).

Before *H. marelatus* can become an effective biological control agent, the most effective time of application should be determined. Entomopathogenic nematodes are ineffective when applied to the foliage because the nematodes quickly desiccate and are killed by UV light (Bélair et al. 1998, Gaugler et al. 1992). Timing of *H. marelatus* is a

critical issue because Colorado potato beetle is a foliage-feeding pest and only soil inhabiting stages are vulnerable (mature larvae entering the soil for pupation, pupae, and newly emerging adults).

The stages targeted by this study were mature fourth instars, pupae, and newly emerged adults while they are in the soil. The objective of the study was to determine the most suitable timing of *H. marelatus* application for effective Colorado potato beetle control in the field.

Materials and Methods

Nematode Rearing. Wax moth larvae, *Galleria mellonella* L., were used as a host for nematode production because the costs are low and maintenance of the wax moth culture is easy. *G. mellonella* were reared at 30 °C in rearing chambers as described by Dutky et al. (1962). The nematode was obtained from Oregon State University, Corvallis, OR and maintained on last instar *G. mellonella* using a modification of the method described by Dutky et al. (1964). *G. mellonella* was kept in a rearing chamber at 20 °C. Nematodes were extracted using the technique described by White (1927) and stored at 4 °C in distilled water aerated by an aquarium pump.

Experimental Design and Nematode Inoculation. The study was conducted in microplots at the Michigan State University Horticulture Farm. The soil was sandy loam soil and each microplot was enclosed with a 2 x 2 m lumite cage (841 μm screen) (Fig. 5.1). In 2000, nine potato tubers (cv. Snowden) were planted on 2 June in each of the twelve cages. Plots were maintained using standard fertilization and herbicide treatments, but no insecticides or fungicides were used. Irrigation, approximately 4 cm/wk, was applied whenever rainfall was inadequate to meet evapotranspiration needs. In 2000,

three egg masses were introduced into each cage. When larvae reached third instar (17 July 2000), numbers were reduced to 5/plant (45/cage). These numbers were chosen to provide adequate numbers without serious defoliation of the potatoes.

Methods were similar in 2001, except that planting date was 21 June and third instars were introduced directly on plants (four/plant, 36/cage, on 18 July 2001).

The arrangement of treatments was completely randomized among cages with three replications/application time. Infective juvenile nematodes were applied to the soil when fourth instars were first present (18 July 2000 and 19 July 2001, 46 d after planting in 2000 and 28 d after planting in 2001), at peak fourth instar (25 July 2000 and 23 July 2001, 53 d after planting in 2000 and 32 d after planting in 2001), or at peak pupation/when most 4th instars had entered the soil (31 July 2000 and 27 July 2001, 59 d after planting in 2000 and 36 d after planting in 2001). Three cages were left without nematodes.

A concentrated solution of nematodes was diluted in 2 L of water to acquire a field rate of 1 billion nematodes/hectare and applied to the soil in each cage using a Chapin (Chapin Manufacturing, Inc., Batavia, New York) single nozzle hand-held sprayer at 207 kPa (Fig. 5.2). During application of nematodes I continually shook the sprayer to ensure uniform nematode mixing. Control plots received water only. Immediately following nematode application, plots were irrigated (4 cm).

Weather data was recorded daily using a nearby weather station maintained by the Michigan State University Agricultural Weather Office. Data collected included maximum and minimum air temperatures, maximum and minimum soil temperatures, precipitation, relative humidity and estimated evapotranspiration.

Beetle Population Analysis. Emerging Colorado potato beetle summer adults were counted 2-3x/week in each cage and each new adult was marked on the elytra with a paint pen (DecoColor Opaque Paint Marker, Uchida of America Corporation, Carson, CA) to ensure that beetles were not counted more than once.

***H. marelatus* Persistence.** At the end of the study in 2000 when cages were disassembled, the plot area under the cages was marked with stakes so that long-term persistence could be evaluated. At the beginning of the 2001 field season, the presence and pathogenicity of *H. marelatus* was assayed by burying five fourth instar *G. mellonella* wrapped in nylon bags approximately 6 cm below the soil (two per cage). The *G. mellonella* larvae were collected 1 wk later and dissected to determine *H. marelatus* infection. This persistence test was conducted weekly from 30 April to 29 May 2001.

Statistical Analysis. Colorado potato beetle numbers were analyzed using SAS general linear model (GLM) and Fisher's protected least square significant difference (SAS Institute 1988).

Results

Beetle Population Analysis. In 2000 and 2001, mean adult emergence was less than 3 beetles/cage at each sampling date in nematode treated plots (Fig. 5.3). Mean numbers emerging in untreated cages were as high as 14/cage and 7/cage in 2000 and 2001 respectively. In 2000, peak adult Colorado potato beetle emergence in untreated cages occurred at 229 Julian days (16 Aug 2000) and beetles had stopped emerging by 232 Julian days (19 Aug 2000). However in 2001, beetles in control cages were emerging continuously throughout the sample period (213 - 230 Julian days; 31 July – 17

Aug) (Fig. 5.3), even though all larvae (3rd instars) were introduced into the cages on the same day.

There was significantly more total adult Colorado potato beetle emergence in untreated plots than in any nematode treated plots in 2000 and 2001 (Fig. 5.4). In 2000, an average of 50% of larvae survived to emerge as adults in control cages. In nematode treated cages there less than 10% of the larvae survived to emerge as adults (Fig. 5.4). All application times were equally effective.

In 2001, there was a higher percentage of emergence from untreated and nematode treated cages than in 2000. Survival of larvae to emerge as adults in control cages was 50% in 2000 and 80% in 2001. In nematode treated cages there was less than 20% survival to emergence (Fig. 5.4). Percent control in 2000 was 61% and 55% in 2001, using Abbott's (1925) formula. Again, all application times were equally effective.

***H. marelatus* Persistence.** All *G. mellonella* larvae recovered from the field were alive or were dead from other unknown infection and no *H. marelatus* was observed after dissection.

Discussion

Beetle Population Analysis. The early instars of Colorado potato beetle feed on the foliage of the potato plant. This means that these larvae are not potential targets for control with *H. marelatus* and that the potato may suffer initial damage from larvae of the first generation of Colorado potato beetle. Crop rotation would be a logical addition to use of nematodes, since crop rotation will delay the arrival of adults and reduce the numbers of first generation larvae. Nematode effectiveness against Colorado potato beetle was likely optimized in our study due to the protection of cages. The soil inside the cages had relatively high moisture compared to soil outside the cages and remained cool throughout most of the season. The cages seemed to create a habitat with optimal soil edaphic factors (Koppenhöfer et al. 1995).

***H. marelatus* Persistence.** This persistence study was designed to determine if *H. marelatus* infective juveniles could survive and retain pathogenicity over the winter in the soil. It is possible that infective juveniles survived the winter but at undetectable numbers, infective juveniles did not survive the winter, or infective juveniles survived but lost pathogenicity or host finding ability. *H. marelatus* survives winters in its place of origin along the west coast of the U.S. including Washington, Oregon and California and so may also be able to survive in Michigan. Winter soil temperatures in Michigan at depths where the nematode might occur do not go below freezing, but the duration of cold temperatures in Michigan may be longer than on the west coast and temperatures may approach freezing or freeze in the upper soil during cold winters in MI. If overwintering survival does occur, then possible negative effects on native soil fauna in Michigan should be taken into consideration for the future use of the nematode.

Colorado potato beetle is the most destructive insect pest of potatoes in North America and one of the most significant agricultural insect pests worldwide. Achieving adequate control of this pest is complicated by its ability to consistently and repeatedly develop resistance to the insecticides used against it.

For effective control of Colorado potato beetle, *H. marelatus* should be applied when Colorado potato beetle is ready to pupate or already in the soil. Mortality induced by *H. marelatus* may ultimately reduce insecticide use in Michigan potatoes and maintain the efficacy of existing products. *H. marelatus* has the potential of becoming part of current integrated pest management programs for sustainable control of Colorado potato beetle. It may also have the potential for management of other insect pests that spend at least a portion of their life cycle in the soil.

Figure 5.1. Each microplot was a 2 x 2 m lumite cage (841 μm screen). Nine potato tubers were planted on 2 June 2000 and 21 June 2001 in each of the twelve cages.

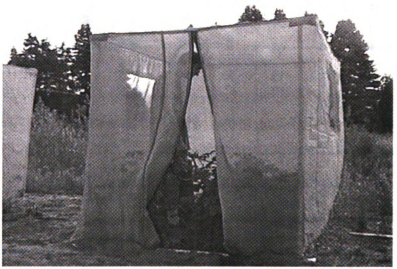

Figure 5.2. A concentrated solution of nematodes was diluted in 2 L of water to acquire a field rate of 1 billion nematodes/hectare and applied to the soil in each cage using a Chapin (Chapin Manufacturing, Inc., Batavia, New York) single nozzle hand-held sprayer at 207 kPa. During application of nematodes the sprayer was continually agitated to ensure uniform nematode mixing.

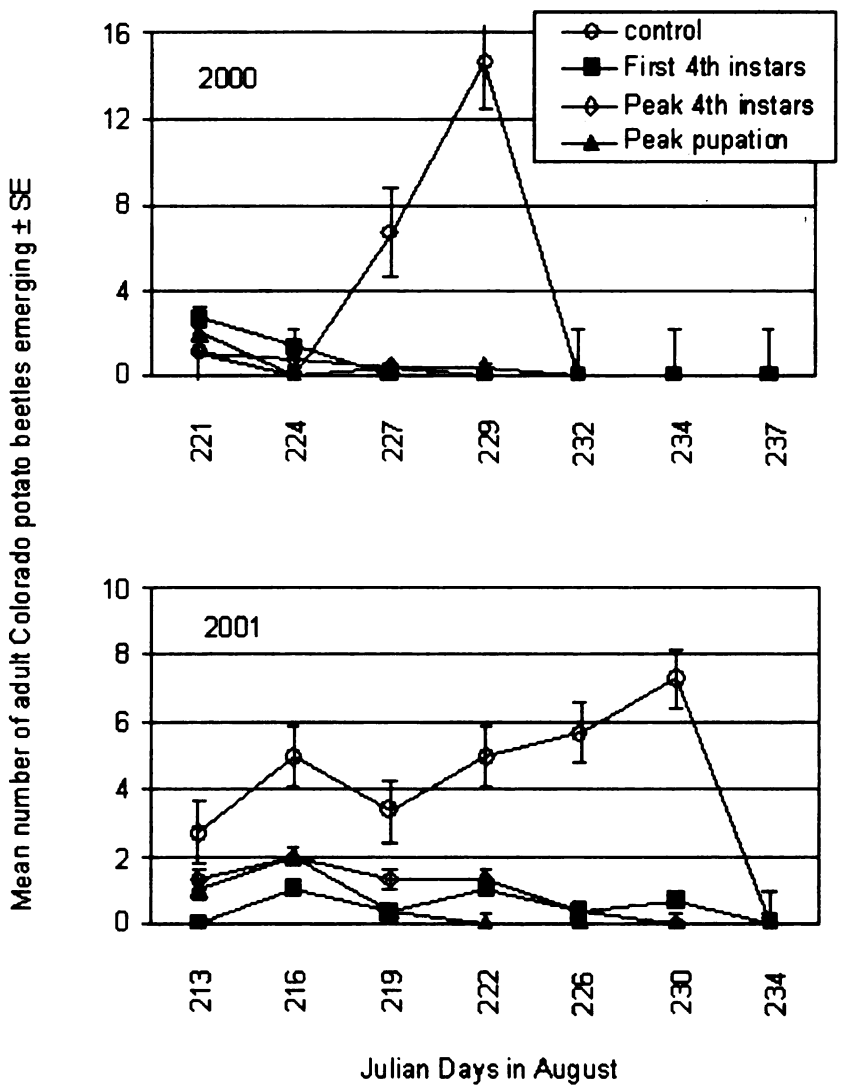

Figure 5.3. Mean numbers of adult Colorado potato beetle emerging vs. Julian days in 2000 and 2001 for untreated plots and treated with *H. mareleatus* at different times.

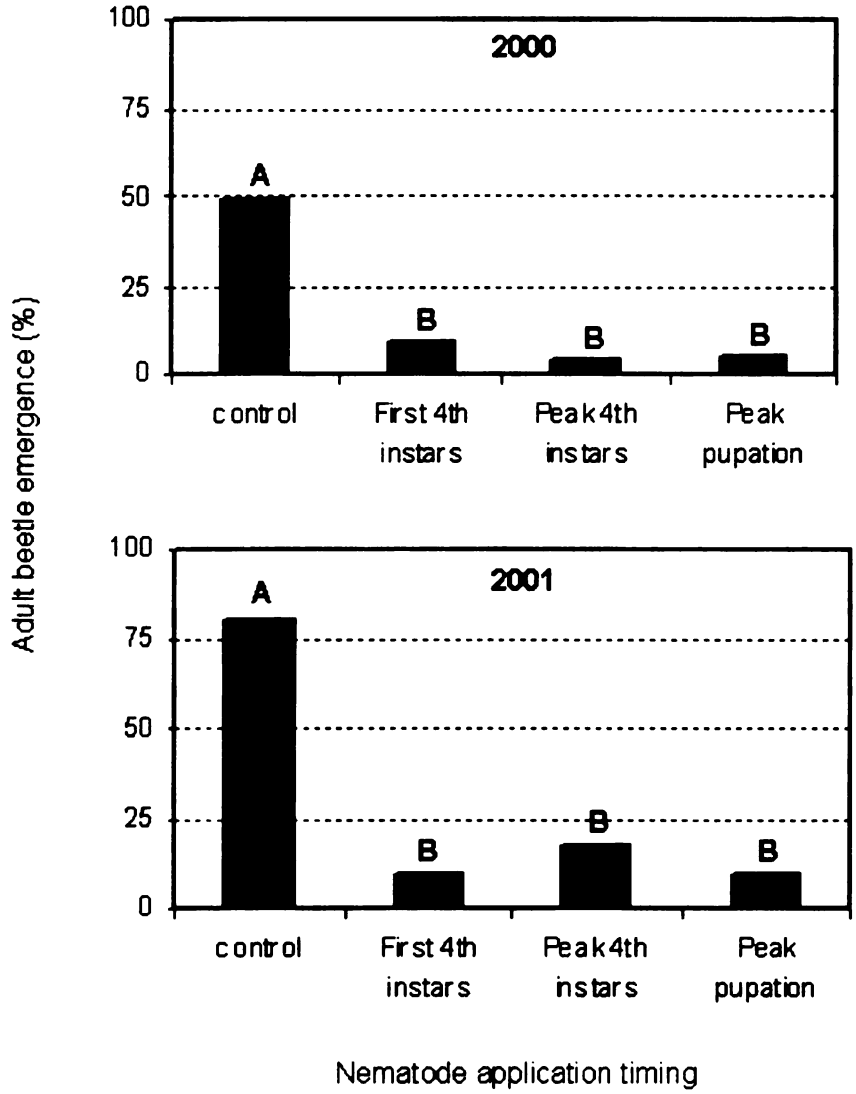

Figure 5.4. Percentage of Colorado potato beetle survival from 3rd instar to summer adult vs. nematode application timing

CONCLUSIONS

Michigan ranks ninth in potato production in the nation (Andresen 2000) and Colorado potato beetle causes up to $14 million in damage annually (Grafius 1997), an economic loss that cannot be overcome with insecticide use alone because the pest has continues to develop resistance to available insecticides (Bishop and Grafius 1996). In the age of environmental awareness, non-chemical alternatives are needed to manage this important pest. That, in turn, requires a broad understanding of the agro-ecological factors that influence the interactions of the target pest and its potential biological agent. This project is the first comprehensive study to investigate the potential of entomopathogenic nematode use in the Michigan potato production system. The project set out to test three specific hypotheses:

Hypothesis 1: Soil type and moisture do not affect the pathogenicity and survival of *H. marelatus* within soil.

Hypothesis 2: Pathogenicity does not differ based on varying rates of *H. marelatus*.

Hypothesis 3: Time of application does not affect control of Colorado potato beetle by *H. marelatus*.

The study of moisture and soil type demonstrated that *H. marelatus* is most effective at high moisture levels and sandy soils. This may be problematic in very dry growing seasons, yet advantageous during seasons with high precipitation. In dry seasons, irrigation should be managed differently after nematodes have been applied. Irrigation must be used for several weeks after application to ensure that soil remains moist enough for nematode survival. Soil type must also be considered before *H.*

marelatus is used for Colorado potato beetle control. *H. marelatus* may be less effective in heavy soil due to lower survival and pathogenicity compared to sandy soil. There may be a need for higher application rates of *H. marelatus* when used in loam.

Based on the information obtained from these studies, soil type and moisture do affect the pathogenicity and survival of *H. marelatus*, thus the hypothesis soil type and moisture do not affect the pathogenicity and survival of *H. marelatus* within soil was disproved.

There were very different results in the two growing seasons testing differing rates of *H. marelatus*. In the first season (2000) with high precipitation, Colorado potato beetle was effectively controlled and *H. marelatus* persisted in the soil throughout the growing season. This may have offered lasting control for fourth instar Colorado potato beetle that continued to burrow in the soil in the month that followed nematode application. In 2001, effective control of Colorado potato beetle was not observed and nematodes did not persist in the soil. It is hypothesized that the dry and hot weather of 2001 is part of the reason for this lack of control. This hypothesis is supported by laboratory results indicating the need for high moisture levels.

Without further tests, it is difficult to say for sure whether the hypothesis, pathogenicity does not differ based on varying rates of *H. marelatus* was disproved. Based on laboratory tests of rates, it seems that *H. marelatus* pathogenicity increases with increased application rate. Rates used in 2000 may have been too high to show any rate effect.

In both 2000 and 2001, timing studies conducted in field cages showed that as long as the stages of Colorado potato beetle found in the soil are targeted, control is

effective. Therefore, the study failed to disprove the hypothesis, time of application does not affect control of Colorado potato beetle by *H. marelatus*.

The development of resistance to most available insecticides in Colorado potato beetle has led to the need for additional control tactics, including biological control. Efforts to control Colorado potato beetle with the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. have been tried with variable success. This, in part, appears to be limited by humidity (Campbell et al. 1985, Feng et al. 1994). Tipping et al. (1999) showed that *Edovum puttleri* Grissell (Hymenoptera: Eulophidae) and *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) is not economically feasible on a large scale for control of Colorado potato beetle in tomatoes. Biological control using *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) has also been attempted. However, Colorado potato beetle eggs are not the optimal diet for *C. maculata* and there is a preference for aphids, when available (Hazzard and Ferro 1991). In order to slow down resistance of Colorado potato beetle to pesticides, there is a need to develop additional management strategies.

When using any management tactic, including biological control, multiple forms of control lead to the best management. I believe that the use of *H. marelatus* needs to be used as part of an integrated pest management program to provide control of Colorado potato beetle that is economically feasible. This is due to the inconsistency of sufficient control, the cost of nematode rearing and the difficulty involved in the application process due to edaphic needs of the nematode.

There is much promise of *H. marelatus* when combined with ecologically sound pest management practices to control Colorado potato beetle. Some of these include the

continuation of resistance management in Colorado potato beetle, crop rotation, plant breeding, cultural controls and minimal insecticide use. Using minimal insecticides might include a half dose of a systemic insecticide at planting with no later applications. This half dose will act more like a full dose in a small potato plant. Assuming that genes in Colorado potato beetle coding for resistance come at fitness or other cost to the beetle, then minimizing and combining controls may actually select beetles that are susceptible to such controls.

Colorado potato beetle was, in its place of origin, an economically insignificant insect that fed on weedy early succession plants of the desert. Colorado potato beetle has changed from the insect that was a part of the ecosystem, to an insect that totally dominated and the potato industry. Confusion over its distribution may have had a lasting impact on mismanagement of the insect as a pest. As with most "introduced" insect pests, humans are to blame for the explosion of Colorado potato beetle in the United States and the world. A clear understanding of the ecology of Colorado potato beetle would have been very valuable information a century ago when assumptions were made that have only recently been corrected.

There are major differences in host preference among geographically isolated populations of Colorado potato beetle (Hsiao 1978). However, since many populations of beetles around the world can still interbreed, technically there is still one species of Colorado potato beetle. Hsiao (1978) observed that Colorado potato beetle in Arizona still prefers a weedy natural host, *Solanum elaeagnifolium*, while beetles in neighboring Utah, New Mexico and Texas found the host unsuitable.

In areas that Colorado potato beetle is considered a major pest problem control efforts often fail. There is a constant battle of insecticide resistance in Colorado potato beetle and no effective biological control agents have been found. However, in its place of origin (including Mexico and the Southwest United States), Colorado potato beetle is found in low population densities and natural enemies seem to play a major role in regulation of beetle populations (Logan et al. 1987). Research done on natural populations of Colorado potato beetle seem to indicate that the beetle, as a pest, should have been dealt with as an exotic or introduced species during the early years that it was considered a pest. Obviously there are problems with how researchers view an exotic pest. Although Colorado potato beetle occurred in a small area of the United States, and politically it was not introduced, the ecology of the insect is completely different in its natural habitat.

There is a clear need for sound ecological science on newly discovered species. Colorado potato beetle is a classic example of a species that was not studied before its status as a pest. As a pest, however, research takes a different turn, focusing on control or eradication rather than a basic approach to understanding the organism. With all of the information currently available on the ecology of Colorado potato beetle, it is surprising to see how researchers continue to lose the battle to control the "pest". Many researchers have focused on ecology and have added a great wealth of knowledge to the literature. However, farmers and applied scientists too often overlook basic research. Colorado potato beetle is well adapted to its environment and when introduced to a new environment, it adapts at a remarkable speed. It is inevitable that Colorado potato beetle will continue its spread until its distribution is worldwide.

Based on the historical mistakes we have made with regard to Colorado potato beetle, we must take every precaution to avoid the same type of disaster with something meant for good. By this, I mean the re-distribution of *H. marelatus*. Humans have been moving organisms around the earth for centuries. It is usually with good intentions: food crops, domesticated animals or biological control of pest species. It is not always a good idea to alter the distribution of natural enemies.

However, *H. marelatus* does not seem to persist in the environment for longer than three months. This is based on sampling results used to measure the persistence of *H. marelatus*. We must also, however, be careful when interpreting this sampling data because often entomopathogenic nematodes occur at such low numbers that traditional sampling does not reveal their presence.

For optimal control of Colorado potato beetle, *H. marelatus* should be used with other forms of control such as crop rotation, resistant varieties of potatoes and pesticides, where necessary. Mortality induced by *H. marelatus* may ultimately reduce insecticide use in Michigan potatoes, reduce selection pressure for insecticide resistance, and maintain the efficacy of existing insecticide products. *H. marelatus* has the potential of becoming part of current integrated pest management programs for continued control of Colorado potato beetle.

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Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2002-12

Title of thesis or dissertation (or other research projects):

Use of the entomopathogenic nematode, *Heterorhabditis marelatus*, to control the Colorado potato beetle, *Leptinotarsa decemlineata*

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums: N/A

Investigator's Name(s) (typed)
Nathan L. Cottrell

Date December 3, 2002

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Leptinotarsa decemlineata	Michigan Montcalm Co. Entrican - Montcalm MSU Research Farm on potato 17-Jul-01 Nathan Cottrell coll.					24			MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Nathan L. Cottrell

Date 3-Dec-02

Voucher No. 2002-12

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 7 MAY 2003

Date