# Evaluation of the potential of *Heterorhabditis megidis* H90 (Nematoda) and its associated bacterium, *Photorhabdus luminescens,* in the control of some forest insect pests

by

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**Title of Thesis/Project/Extended Essay**

Evaluation of the potential of Heterorhabditis megidis H90 (Nematoda) and its associated bacterium, Photorhabdus luminescens, in the control of some forest insect pests.

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## ABSTRACT

The entomopathogenic nematode, *Heterorhabditis megidis* Poinar, Jackson, and Klein, 1987 and its mutualistic bacterium, *Photorhabdus luminescens* (Thomas and Poinar 1979) Boemare, Akhurst, and Mourant comb. nov., are biological control agents of insect pests. Heterorhabditids have unique host seeking and reproductive strategies and have anatomical characteristics that encourage consideration of their application in insect control programmes. An isolate, *H. megidis* H90, obtained from the Okanagan Valley, British Columbia, was evaluated in laboratory and greenhouse experiments for infectivity and efficacy against several forest insect pests. Some comparative experiments were conducted using the nematode *Steinernema carpocapsae* strain All obtained as Biosafe®, a product of Biosys, Palo Alto, California.

Results from Petri plate tests using larvae of the greater wax moth, *Galleria mellonella* (a nematode-susceptible test insect), showed that temperature influences the number of, and speed at which nematodes enter the insect, and development within the insect. *Compared to H. megidis, S. carpocapsae* had a faster infection rate. $LT_{50}$ values in Petri plates ranged from 42 h at 24 °C to 181 h at 12 °C for *H. megidis* and similar values were obtained for *S. carpocapsae*. Although infectivity from both *H. megidis* and *S. carpocapsae* occurred at 8 °C, neither nematode reproduced at this temperature. There was no multiplication of *P. luminescens* occurred at 8 °C. In laboratory experiments, *H. megidis* reacted positively to chemical cues from a target host.

Petri plate experiments involving the Douglas-fir cone gall midge, *Contarinia oregonensis* (Foote) and the spruce cone maggot, *Strobilomyia appalachensis* Michelsen, resulted in 27 and 56 % mortality, respectively. Mortality was influenced by the temperature, dosage, and the developmental stage of the insect. In Petri plate experiments using eastern spruce budworm, *Choristoneura fumiferana* Clemens, and root weevils, *Otiorhynchus ovatus* L., mortality of 100 and 30 % respectively, was obtained. Experiments using fungus gnats, *Bradysia* sp., resulted in 91% mortality in Petri plates, and a minor decrease in the number of adults which were trapped under greenhouse conditions.

The results of these experiments are sufficiently encouraging to justify further research on the application of entomopathogenic nematodes against forest insect pests. To do so will require nematode rearing facilities and the identification of appropriate test sites. The potential to develop large scale production facilities for *Heterorhabditis* spp. in Canada is great.

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## 1.0 INTRODUCTION

In 1929, while working to control the newly introduced beetle pest, *Popillia japonica* Newm., R. W. Glaser of the Rockerfeller Institute, Princeton, New Jersey, and G. Steiner of the United States Department of Agriculture discovered a parasitic nematode that they later described as *Neoaplectana glaseri* Steiner (Glaser, 1932; Steiner, 1929) which is now known as *Steinernema glaseri*. This finding initiated the study of entomopathogenic nematodes in North America and this field of research is now world-wide. Although much research was done on entomopathogenic nematodes over the next few decades, progress on their effective use as biological control agents was slow. *Heterorhabditis,* a nematode genus with a biology similar to *Steinernema,* was not described until 1975 (Poinar, 1975). Kaya and Gaugler (1993) list three species in this genus, namely, *H. bacteriophora* (=*heliothidis*) Poinar, *H. megidis* Poinar, Jackson, and Klein, and *H. zealandica* Poinar. *H. megidis* is distinguished from *H. bacteriophora* and *H. zealandica* by DNA analysis and morphological features which include the length of the infective juvenile, the distance from the head to the excretory pore, and the bursa of the males (Poinar *et al.*, 1987). *Heterorhabditis* spp. are distributed world-wide (Poinar, 1990), and recently, were specifically reported from western Canada (Mracek and Webster, 1993).

The nematode families Heterorhabditidae and Steinernematidae differ from most other rhabditids by having a mutualistic association with specific bacteria which they release into insects. Steinernematids and heterorhabditids carry bacterial symbionts belonging to

the genera *Xenorhabdus* (Enterobacteriaceae) (Akhurst, 1983), and *Photorhabdus* (Enterobacteriaceae), respectively. The bacteria occur throughout the intestine of infective juveniles (IJ's) (Endo and Nickle, 1991). The third stage juvenile, or dauer larva, is the infective, non-feeding, stage. The IJs of both families are attracted to insects which they enter via the mouth, anus, or spiracles, and then penetrate into the hemocoel, where they release the bacteria (Poinar, 1979). However, heterorhabditids are unique in their possession of an anterior tooth which enables them also to penetrate the cuticle and intersegmental membranes of their host (Bedding and Molyneux, 1982). Another distinguishing attribute is that the third stage dauer larva of heterorhabditids is tightly ensheathed in the second stage cuticle which is not easily removed, whereas the dauer larva of steinernematids has a loosely fitting, second stage cuticle that is easily lost during movement in the soil, thereby increasing its susceptibility to infection by soil organisms (Kaya, 1990). Once in contact with a potential insect host, the IJs move over the insect's cuticle for several minutes to several hours before attempting penetration (Bedding and Molyneux, 1982). The principal target organ of the nematode in the insect is the fat body (Milstead, 1979a). Upon penetration, the nematodes evade hemocyte recognition, by some unknown mechanism (Dunphy and Webster, 1988). Failure of the nematode to suppress the initial host response ensures that any contaminating bacteria which entered the wound site would be removed. The hemocytotoxic activity which subsequently occurs is caused by the fatty acids of lipid A, a constituent of the outermost layer of the wall of the bacteria (Dunphy and Webster, 1988). Normally, death of the host occurs about 48 h after nematode penetration (Kaya, 1990).

The bacteria establish favorable conditions for their own reproduction and that of the nematode by providing nutrients and producing antibiotics (Akhurst,1986). Upon death of the host, the IJs feed to maturity on the bacteria. Heterorhabditid IJ's develop into hermaphroditic females, whereas steinernematids develop into males or females (Poinar, 1990). The second generation of heterorhabditids (progeny of the hermaphroditic females) consists of amphimictic females and males (Poinar, 1990). After mating, the eggs hatch inside the females and develop to the second or even third infective stage before emerging from the female (Poinar, 1975). The IJs leave the host when the cadaver cuticle breaks down, and enter the soil where they remain until another host is found or they perish (Popiel and Hominick, 1992).

These rhabditids are referred to in various ways in the literature: parasitic nematodes, entomogenous nematodes, entomopathogenic nematodes, entomoparasitic nematodes, entomophagous nematodes, insect-nematodes, and entomophilic nematodes. I prefer the term entomopathogenic. In the nematological literature, there is contradiction and misunderstanding in the use of terminology describing concepts of pathogenicity and virulence. Pathogenic ['pathos' (Greek) = suffering] can be considered a comprehensive term that refers to the ability to cause disease. Terms such as virulence, avirulence, and aggressiveness, are used to qualify and quantify pathogenicity. I consider virulence, the observable effects of a pathogen (Holliday, 1989), to be the best term to describe degrees of pathogenicity.

Since the discovery of *Steinernema glaseri* in 1929, the use of entomopathogenic nematodes has developed to the point at which commercial formulations of *Steinernema*

spp. are readily available in many countries world-wide. Biosys (Palo Alto, CA.) is the largest nematode-producing company in the world, with products aimed at lawn and garden insects (Biosafe®) , larvae of root weevils and girdlers in cranberries (Biosafe-N®), and turf and ornamental nursery pests, citrus weevil, and artichoke plume moths (Biovector®). Together with Ciba-Geigy, Biosys markets a product "Exhibit", for control of turf and ornamental pests. Other North American nematode products include Nematec (Bioquest, San Leandro, CA), Scanmask™ (Biologic, Willow Hill, PA), Bioguard™ (Gardenville of Texas, TX), Guardian Nematodes™ , Hydro-Gardens, Inc. (Colorado Springs, CO), and Dart ™ (Praxis , Allegan, MI). Internationally, there are commercial products available in England, Italy, The Netherlands, Sweden, Australia, Switzerland, and Thailand (Poinar, 1992). In Canada, there are no purely commercial rearing facilities (Speranzini, 1992). Although heterorhabditids may be more virulent than steinernematids (Bedding, 1981; Bedding *et al.*, 1983; Dunphy and Webster, 1986) commercial formulations of heterorhabditids are not readily available in Canada.

The taxonomy of the genus *Heterorhabditis* is more challenging than *Steinernema* because of the absence of convenient morphological characters and difficulty of testing mating compatibilities as the result of the male's fragility (Popiel and Hominick, 1992). Molecular techniques can be used to supplement morphological information in separating and identifying species (Akhurst,1987; Curran and Webster, 1989). Nematodes of the same species which consistently differ in biological attributes such as virulence, host finding ability, persistence and tolerance to environmental conditions are recognized as strains (Kaya and Gaugler, 1993). Poinar (1990) listed 23 strains of *H. bacteriophora,*

six of *H. zealandica* and one of *H. megidis* (Table 1). Two additional strains of *H. megidis* have since been isolated: *H. megidis* H90 from Canada (Dr. J. M. Webster, Simon Fraser University, Burnaby, B. C., pers. comm.), and *H. megidis* HNA from the U.S.A. (Wright, 1992).

Poinar and Thomas (1965) described the bacterial symbiont of *Steinernema carpocapsae* Weiser (strain DD136) as a new species "*Achromobacter nematophilus*". The genus *Achromobacter* was subsequently rejected, and Thomas and Poinar (1979) erected a new genus *Xenorhabdus* within the Enterobacteriaceae, to accommodate the symbionts of *Steinernema* and *Heterorhabditis* spp. They described two species in this genus, *Xenorhabdus nematophilus* Poinar and Thomas (Thomas and Poinar, 1979) and *X. luminescens* Thomas and Poinar (Thomas and Poinar, 1979). These species are symbiotic with *Steinernema* spp. and *Heterorhabditis* spp. , respectively. A molecular study of the relatedness of *Xenorhabdus* spp. caused Boemare *et al.* (1993) to propose a new genus, *Photorhabdus,* to accommodate *X. luminescens.*

*Photorhabdus luminescens* are gram-negative, asporogenous, rod-shaped bacteria. The cell size is highly variable within and between cultures, being 2 x 0.5 to 10 x 2 $\mu$m with occasional filaments up to 30 $\mu$m long; spheroplasts occur in the last stage of exponential growth. Cells of *P. luminescens* are motile with peritrichous flagellation, and are facultatively anaerobic. *Photorhabdus luminescens* is distinguished from *Xenorhabdus* spp. by their luminescence and catalase activity (Boemare *et al.* , 1993).

*P. luminescens* occurs in two forms (Akhurst, 1980), which differ in their production of antibiotics and lecithinase and in their absorption of dyes. Nematodes reproduce

Table 1. Species and Strains of *Heterorhabditis* (Poinar, 1990)

| Species | Strain | Geographical Locality |
|---|---|---|
| *bacteriophora* Poinar 1976 | HB1 | Brecon, South Australia |
| (=*heliothidis* (Khan, | NC1 | Clayton, NC, U.S.A. |
| Brooks, and Hirschmann, | C8406 | Hailing Island, Guangdong, China |
| *1976*) | It145 | Forli, Italy |
| | V16 | Geelong, Victoria, Australia |
| | NC14 | Clayton, NC, U.S.A. |
| | NC69 | Raleigh, NC, U.S.A |
| | NC127 | Lewiston, NC, U.S.A. |
| | NC 162 | Rocky Mount, NC, U.S.A. |
| | Arg1 | Rio Cuarto, Argentina |
| | Hamm | Bryon, GA, U.S.A |
| | Forschler | Lexington, KY, U.S.A. |
| | Ali | Apple Valley, CA, U.S.A. |
| | Overholt | Dimmit, TX, U.S.A. |
| | McCoy | Plymouth, FL, U.S.A. |
| | Schroeder | Orlando, FL. U.S.A. |
| | Brazil | Pernambrico, Brazil |
| | Creighton | Charleston, SC, U.S.A. |
| | NC200 | Reidville, NC, U.S.A. |
| | NC 323 | Salisburg, NC, U.S.A. |
| | NC 405 | Clinton, NC, U.S.A. |
| | NC 476 | Whiteville, NC, U.S.A. |
| | HP 88 | Logan, UT, U.S.A. |

Table 1. Species and Strains of *Heterorhabditis*. (continued)

| species | Strain | Geographical Locality |
|---|---|---|
| *megidis* Poinar, Jackson, | HO1 | Ohio, U.S.A |
| and Klein, 1987 | H90 | Okanagan Valley, B.C, Canada |
| | HNA | U.S.A. |
| *zealandica* Poinar | HLit | Lithuania |
| [=New Zealand | HNach | Nachodka, Russian Federation |
| population | HQ614 | Bundaberg, Queensland, Australia |
| of *H. heliothidis* sensu | T310 | Sandy Bay, Tasmania, Australia |
| Wouts (1979)] | T327 | Nicholl's Rivulet, Tasmania, Australia |
| | NZH, NZ | Auckland, New Zealand |

faster and more prolifically in the presence of the primary form (Bedding, 1981) which is unstable and occurs in mature, IJ nematodes and in the insect hemocoel. The secondary form is usually stable, is sometimes produced within the host cadaver, and inevitably occurs on artificial media if colonies are infrequently subcultured. *Photorhabdus luminescens* kept on artificial media changes from primary to secondary phase as early as 6 d after being cultured (Chen, 1992). On nutrient agar, colonies of the primary form of *P. luminescens* are white, mucoid, convex and circular with an irregular margin; the colonies of the secondary form are translucent, and are flatter and larger in diameter than the primary form (Akhurst, 1983). On nutrient agar supplemented with bromthymol blue (BTB) and triphenyltetrazolium chloride (TTC) (NBTA medium) , primary form colonies of *P. luminescens* are greenish to reddish brown overlaid by dark blue and are surrounded by cleared zones in the agar caused by the adsorption of BTB. The reddish color of the primary form bacteria occurs 3-5 d after culturing, and is caused by the adsorption and reduction of TTC. There is no clear zone around the secondary form colonies because they lack the ability to adsorb BTB. The secondary form is usually reddish to rusty because of the adsorption and reduction of TTC (Woodring and Kaya, 1988). In *P. luminescens*, bioluminescence is stronger in the primary than in the secondary form (Boemare and Akhurst, 1988). Like *Xenorhabdus* spp., *P. luminescens* is highly pathogenic to insects; the $LD_{50}$ [1] is < 100 cells when these bacteria are injected into the haemocoel of *Galleria mellonella* (L.).

[1] $LD_{50}$ and $LT_{50}$ respectively refer to the lethal dose required to kill 50% of a population, and the time required to kill 50% of a population at a given dose.

The temperature ranges over which steinernematids and heterorhabditids develop and reproduce differ between species and strains (Molyneux, 1983). The effect of temperature on $LT_{50}$ and $LD_{50}$ of host insects has been reported for many strains of *Heterorhabditis* and *Steinernema.* Among *S. feltiae* CA, *S. feltiae* AKLD, *S. carpocapsae* OHIO, *Heterorhabditis* sp. HL81, *H. bacteriophora* V16, *H. zealandica* HNZ, and *H. megidis* HNA, all grew and reproduced at temperatures > 10 °C, but only *S. feltiae* strains CA and AKLP reproduced at 10 °C (Wright, 1992). Wright (1992) also reported reported that the rate of reproduction at 10 °C for the different nematode strains was directly related to the rate of growth of the bacterial symbiont at 10 °C. Zervos *et al.* (1991) reported that no juvenile *H. heliothidis* emerged from *G. mellonella* kept in Petri plates treated with inocula of 5 - 500 IJs/plate for 60 d at 10 °C.

Generally, heterorhabditids have a greater host range than steinernematids, but in terms of mobility, infectivity, and reproduction, they are limited by cool temperatures to a greater extent than steinernematids (Molyneux, 1983). Wright (1992) proposed that by rearing axenic heterorhabditids in media inoculated with the cold active bacteria of *S. feltiae* , an improved biocontrol agent might result. Gerritsen and Smits (1993) interchanged four strains of *P. luminescens* between the associated four strains of *Heterorhabditis* sp., and although the ability of the heterorhabditids to grow and multiply on different bacterial isolates was demonstrated, in no instance was pathogenicity enhanced. Han *et al.* (1991) interchanged several *P. luminescens* isolates among several heterorhabditids and found that no heterorhabditid strain developed on every bacterial isolate, although the symbiont from *H. megidis* seemed to be the most universally

accepted. If development occurred, the bacterial isolate influenced yields of nematodes which were sometimes greater when the natural bacterial symbiont was replaced with one from a different nematode strain. *P. luminescens* isolates do not support the culture of any *Steinernema* sp. *in vitro* (Akhurst,1983) . Heterorhabditids and steinernematids can reproduce and develop when associated with several common bacteria, but reproduction is severely limited when compared with that achieved with the natural symbiont (Boemare and Akhurst, 1990). Attempts have been made with only transitory success to acclimatize steinernematids and heterorhabditids to low temperatures (Burman and Pye, 1980). Dunphy and Webster (1986) reported that *in vitro* culturing of *H. heliothidis* at 20 and 25 °C for 9 months did not alter the $LT_{50}$'s at the respective temperatures; nematodes failed to emerge from an attempted third passage at 15 °C.

The virulence of entomopathogenic nematodes is a function of the associated bacteria, the strain of nematode used, the host, and the prevailing environmental conditions. Investigations into host-parasite interactions may reveal why virulence varies in different environments. Factors such as the speed of penetration, number of nematodes per insect, number of bacteria in the haemocoel, and timing of bacterial release remain unexamined.

Host selection by IJs may be considered in four stages: search, attachment, recognition, and host penetration (Kaya and Gaugler, 1993). Searching by heterorhabditid IJs is an active process that occurs in response to host-associated chemical and physical stimuli (Kaya, 1990), e.g. $C0_2$ (Gaugler *et al.*, 1980), excretory products (Schmidt and All, 1978, 1979), and temperature gradients (Pye and Burman, 1981). Lewis *et al.* (1992a) described *S. glaseri* as a cruising species that moves through the

soil in search of hosts. *S. carpocapsae*, by contrast, is an ambushing species that waits for a host to come to it. After exposure to relevant host cues, cruisers often switch from long range to localized search (Bell, 1990), whereas ambushers rely on contact with the host.

Milstead (1979b) reported the $LD_{50}$ for killing *G. mellonella* larvae with *H. bacteriophora* as 3-6 IJ's per larva. Topical exposure to the associated bacteria had no effect on larval mortality and *per os* delivery of the bacteria caused only 7% mortality. When the bacteria were injected into the haemocoel, 100% mortality was achieved with < 15 bacteria per larva (Milstead, 1979b). The bacteria facilitate reproduction by the nematode by providing nutrients and suppressing the growth of contaminating organisms (Poinar and Thomas, 1966), e.g. *P. luminescens* produces antimicrobial metabolites, such as hydroxystilbene and dithiolopyrrolone derivatives (McInerney *et al.*, 1991).

The nematode vectors act as "living syringes" for the bacteria. Pathogenesis apparently also involves several toxins produced by both the bacteria and the nematode. A single axenic nematode can kill a *G. mellonella* larva (Poinar and Thomas, 1966). Extract from nematode inhabited growth medium was toxic when injected into insect larvae (Poinar, 1979). An insecticidal factor, possibly an excretory or secretory product of the third and fourth stage juveniles, has been found in axenic *G. mellonella* parasitized by axenic *S. feltiae* (Burman, 1982).

McInerney *et al.* (1991) reported that an antibiotic, xenorhabdin 2, produced by *Xenorhabdus* spp., had *per os* larvicidal activity against *Heliothis punctigera* Hall. Extracellular metalloproteases are produced by several species of insect pathogens,

including *P. luminescens* (Schmidt *et al.*, 1988), and may cause death by precipitating hemolymph proteins and damaging the haemocytes (Lysenko, 1985). Ensign *et al.* (1990) discovered a potent insecticidal toxin produced during growth of *P. luminescens* in larval tomato hornworms, *Manduca sexta* (L.) One nanogram of purified toxin injected into fifth instars caused death in 12-24 h. The toxin was not fatal when ingested and did not show protease or phospholipase activities (Ensign *et al.*, 1990). Toxic compounds were produced in *G. mellonella* larvae infected with *H. bacteriophora* almost immediately after the released bacteria entered the host hemocoel (Jarocz *et al.* 1991). By injecting larval extracts from parasitized *G. mellonella,* or spent broth media inoculated with the primary form of *P. luminescens*, into live *G. mellonella* larvae, Jarocz *et al.* (1991) achieved 98% and 100% mortality in 13 and 11 d, respectively. Neither toxic lesions nor larval mortality occurred after ingestion of either the larval extract or the spent broth media. They reported also that the larvicidal activity of *X. nematophilus* was weaker than that of *P. luminescens,* and occurred only when the toxins were present in the hemocoel. Insecticidal activity of the larval extracts coincided with increased proteolytic activity.

As early as the 1960's, *Steinernema* sp. (DD-136) was evaluated as a biocontrol against 12 forest insects (Schmiege, 1963). Examples of studies since 1963 of interactions between entomopathogenic nematodes and forest insects using Schmiege's (1963) susceptibility ratings, are summarized in Table 2. In Canada, the 'Insect-Parasitic

Table 2. Examples of the response of forest insect pests to entomopathogenic nematodes.

| Order | Common and Scientific Name | Stage[a] | Susceptibility[b] | Nematode[c] | Reference |
|---|---|---|---|---|---|
| Lepidoptera | European pine shoot budworm, *Rhyacionia buoliana* (Schiff.) | L | V | DD-136 | Schmiege, 1963 |
| | Eastern Spruce budworm, | L | V | DD-136 | Schmiege, 1963 |
| | *Choristoneura fumiferana* Clemens | P | M | *Hh* | Finney and Bennett, 1982; Finney *et al.*, 1982 |
| | | | | DD-136 | Schmiege, 1963 |
| | Jack pine budworm, | L | V | DD-136 | Schmiege, 1963 |
| | *Choristoneura pinus* Freeman | P | M | DD-136 | Schmiege, 1963 |
| | Red-humped oakworm, *Symmerista albifrons* (A. & S.) | L | M | DD-136 | Schmiege, 1963 |
| | Spruce bud moth, *Zeiraphera canadensis* Mut. & Free. | L | V | *Hh* | Turgeon and Finney-Crawley, 1991<br>Eidt and Dunphy, 1991 |
| Hymenoptera | Introduced pine sawfly, *Diprion similis* (Hartig) | L | V | DD-136 | Schmiege, 1963 |
| | Red-headed pine sawfly, *Neodiprion lecontei* (Fitch) | L | V | DD-136 | Schmiege, 1963 |
| | Yellow-headed spruce sawfly, *Pikonema alsakensis* (Rohwer) | L | V | DD-136 | Schmiege, 1963 |

Table 2. Examples of the response of forest insect pests to entomopathogenic nematodes. (continued)

| Order | Common and Scientific Name | Stage[a] | Susceptibility[b] | Nematode[c] | Reference |
|---|---|---|---|---|---|
| Hymenoptera | Larch sawfly, | L | V | DD-136 | Schmiege, 1963 |
| | *Pristiphora erichsonii* (Hartig) | L | V | *Hh* | Finney & Bennett,1983 |
| | | PP | M | DD-136 | Schmiege, 1963 |
| | | | | | Webster & Bronskill, 1968 |
| | Mountain ash sawfly | L | V | *Hh* | Finney & Bennett, 1983 |
| | *Pristiphora geniculata* (Htg.) | | | | |
| | Birch sawfly | L | V | *Hh* | Finney & Bennett, 1983 |
| | *Arge pectoralis* (Leach) | | | | |
| | European pine sawfly | L | V | *Hh* | Finney & Bennett, 1983 |
| | *Neodiprion sertifer* (Geoffrey) | P | R | *Hh* | |
| Homoptera | Saratoga spittlebug, | | | | |
| | *Aphrophora saratogensis* (Fitch) | N | R | *Hh* | Schmiege, 1963 |
| Coleoptera | White-pine weevil, | A | R | DD-136 | Schmiege, 1963 |
| | *Pissoides strobi* (Peck) | | | | |
| | Pine root collar weevil, | | | | |
| | *Hylobius radicus* Buch. | A | M | DD-136 | Schmiege, 1963 |
| | White spotted sawyer, | L | M | DD-136 | Schmiege, 1963 |
| | *Monochamus scutellatus* (Say) | P | M | DD-136 | |

Table 2. Examples of the response of forest insect pests to entomopathogenic nematodes. (continued)

| Order | Common and Scientific Name | Stage[a] | Susceptibility[b] | Nematode[c] | Reference |
|---|---|---|---|---|---|
| Diptera | Spruce cone maggot, *Strobilomyia* spp. | L<br>P | M<br>R | *Sc* | J. Sweeney (*pers. comm.*)[d] |

[a] L= Larvae, N= Nymphs, PP= Pre-pupae, P= Pupae, A=Adults
[b] Susceptibility ratings based on those of Schmiege (1963): V=Very susceptible, M=Moderate, R=Resistant
[c] DD-136=*Steinernema* spp., *Hh= Heterorhabditis heliothidis, Sc= Steinernema carpocapsae*
[d] Research Scientist, Canadian Forestry Service, Fredericton, N.B.

Nematode Working Group' comprising government, industry, and academia meets annually to discuss progress in the use of nematodes in agriculture and forestry. The founding chairman, Dr. Douglas Eidt, has now been succeeded by Dr. Graham Thurston, Canadian Forest Service, Fredericton, New Brunswick.

Entomopathogenic nematodes could potentially be used as biocontrol agents of forest insect pests in seed orchards, nurseries, and established plantations. With these settings in mind, I selected several representative forest pests for evaluation of *Heterorhabditis megidis* H90 as a biocontrol agent. The insects are of economic importance, have differing life cycles, and represent three orders.

Fungus gnats, *Bradysia* spp. (Diptera: Sciaridae), occur in commercial greenhouses and, under favorable conditions, may develop large populations. Larval fungus gnats damage seedlings roots, and the adults may possibly vector pathogenic fungi, e.g. *Botrytis* and *Fusarium* spp. (Sutherland *et. al.*, 1989). Biosafe® and Biovector®, marketed by Biosys, are advertised as effective biocontrol agents against fungus gnats. These products are used in a provincial forest greenhouse in Orono, Ontario and are considered to be effective against fungus gnat larvae, *Bradysia* spp. when applied through the irrigation system ( C. Staples, pers. comm. , Ministry of Natural Resources. Orono, ON ). In bare root nurseries , larval root weevils, *Otiorhynchus* spp., can seriously damage tree seedling roots (Sutherland *et. al.*, 1989). Being soil dwellers for most of the year, these larvae are good candidates for control by entomopathogenic nematodes in the fall and spring when the temperatures are favorable (Ingraham, 1991). Biosys lists root weevils as insects that may be controlled with Biosafe®, Biovector® and

Helix®.

Many cone and seed insects spend a part of their life in the soil and are difficult to control. The Douglas-fir cone gall midge, *Contarinia oregonensis* (Foote), an important pest in Pacific Northwest seed orchards (Dombrosky and Schowalter, 1988; Miller and Ruth, 1989), spends almost 4 months as a larva and pre-pupa in the duff making it vulnerable to soil dwelling parasites like *Heterorhabditis* spp. The spruce cone maggot, *Strobilomyia appalachensis* Michelsen, has somewhat similar habits (Sweeney and Turgeon, 1994). Females lay eggs singly on or near the seed cones of black spruce, *Picea mariana* (Mill.) B.S.P., shortly after pollination and the larvae progress through three instars before the mature larvae leave the cone in early summer and drop to the ground. These larvae move into the duff, overwinter in the soil as pupae, and adults either eclose the following spring or remain in diapause for another year or more (Hedlin, 1973).

Several budworms, *Choristoneura* spp., feed on forests throughout North America; they are particularly destructive in Canada (Moody, 1993). The eastern spruce budworm, *C. fumiferana*, feeds mainly on balsam fir, *Abies balsamea* (L.) Mill., but eats also red spruce, *Picea rubens* Sarg., white spruce, *P. glauca* (Moench) Voss, black spruce, eastern hemlock, *Tsuga canadensis* (L.) Carr., and tamarack, *Larix laricina* (Du Roi) K. Koch.

Although entomopathogenic nematodes are pathogenic against budworm larvae (Finney *et al.*, 1982; Finney and Bennett, 1984), they have yet to be used in any budworm control program. Budworms have no soil-inhabiting stage. Egg masses are laid on the undersides of needles in the fall. The larvae hibernate without feeding in the

autumn, feed on expanding vegetative buds in the spring, and pupate among the loose webs on the twigs (McGauley and Kirby, 1985).

For most laboratory studies, *in vivo* propagation of nematodes in late instar *G. mellonella* larvae can produce approximately 30,000-50,000 IJs per larva (Woodring and Kaya, 1988). Zervos *et al.* (1991) found that the number of juvenile *H. heliothidis* emerging per *G. mellonella* larva was inversely related to the number of IJs to which the host was exposed. This *in vivo* method may be used to provide nematodes for small scale field application, but is labor intensive and prohibitively expensive, at an estimated cost over two decades ago of $1.00 (U.S) per million nematodes (Poinar, 1972). To treat one hectare of soil for control of black vine weevil in strawberry fields would require 2000 million IJs reared from 40,000 *G. mellonella* larvae (Bedding, 1986).

For large scale field application, it is thus necessary to use *in vitro* procedures. *In vitro* procedures began in the 1940's with the production of axenic and monoxenic cultures. Examples of the artificial media used are: pulp of veal infused with preservatives (Glaser, 1940); raw kidney (Glaser *et al.*, 1942); and dog biscuit (House *et al.*, 1965). The methods were laborious and the yields low (Poinar, 1972). Early *in vitro* production was two dimensional in media laid out in trays. A significant advance in technology was made by using inert foams which were coated with media, increasing the surface area to the volume ratio (Bedding, 1986). Using lipid agar (Wouts, 1981) as a standard, Dunphy and Webster (1989) significantly increased the yield of *H. heliothidis* by modifying the media to provide optimal physical and chemical characteristics. Entomopathogenic nematodes reproduce poorly or not at all if their associated bacterium

is not present (Bedding, 1986). Monoxenic liquid culture of entomopathogenic nematodes is currently being developed, but the technology is proprietary (Popiel and Hominick, 1992). At present, *S. carpocapsae* are routinely produced in 15,000 L fermenters (Popiel and Hominick, 1992). Kaya and Gaugler (1993) hypothesize that the development of a flowable concentrate could revolutionize the formulation and applications of nematode-based insecticides.

Although there are few available commercial products based on *Heterorhabditis* spp., successful production on a commercial scale has been achieved (Wouts, 1981; Bedding, 1984; Popiel and Hominick, 1992). Bedding (1984, 1986) reported a method of producing up to 1700 million IJs of heterorhabditids in a 3 kg bag that was suitable for industrial use. This method is used in China (Bedding, 1990), but high labor costs preclude its use in North America (Freidman, 1990). A comparison of the cost of treating 1 ha of root weevil-infested strawberries with five types of nematode formulations and two synthetic pyrethroids is given in Table 3. Clearly, Bedding's (1984, 1986) methods are approaching the realm of cost effectiveness.

However, other persistent problems with large scale production further preclude the delivery of any commerical formulation. For example, heterorhabditids can only be produced in fermenters that are $\leq$ 7570 L (Georgis 1992). Production in a fermenter with a capacity of 50,000 L would exceed $10,000, with only a 50% chance of successful rearing. In addition, heterorhabditids are more active than steinernematids. The correspondingly high oxygen demand requires that they be kept in small containers, which is cumbersome if large areas are to be treated (Georgis, 1992).

Table 3. Cost of treating one hectare of soil with various nematode based and synthetic pyrethroid insecticides against strawberry root weevil.

| Insecticide | Formulation | Unit cost | Cost per ha.(CAN$) |
|---|---|---|---|
| *Steinernema carpocapsae* All | Biosafe® (covers 20 $m^2$) | $15.00 per box | $7500.00[a] |
| *Steinernema carpocapsae* All | Biovector® (250 million IJs) | $65.00 per jar | $780.00[a] |
| *Steinernema carpocapsae* All | Helix® (3 billion IJs) | $645.00 per box | $645.00[a] |
| *Heterorhabditis megidis* H90 | Bedding Flask Method (50 million per L flask, 120 flasks required[b] per ha.) | $0.02 per million | $60.00 |
| *H. megidis* H90 | Bedding Culture Bag (1.0 -1.7 billion/ bag, 6 bags required[b] per ha.) | $0.01 per million | $30.00 |
| Permethrin[c] | Ambush 500 EC PCP #14882 (70 mL per ha.) | $107.65/L | $21.53 |
| Fenvalerate[d] | Belmark 300 PCP #17873 (200 mL per ha.) | $127.65/L | $25.53 |

[a]Application rate (3 billion infective juveniles per ha) is that recommended on the labels of each individual product of Ciba-Geigy Canada Ltd. Mississauga, Ontario.

[b]Application rate (6 billion per ha) is that suggested by Miller and Bedding (1982). There are no commercially available sources of heterorhabditids in Canada. The cost of raw materials needed for in-house production of nematodes, (excluding labour costs) is that reported by Bedding (1984).

[c] ICI Chipman, Stoney Creek, ON.

[d] Dupont, Toronto, ON.

Immobilization is necessary to prevent the nematodes from depleting their energy reserves (Kaya and Gaugler, 1993). Immobilization through storage at 5-15 °C in aqueous solutions, is feasible but not commercially viable. Consequently, carriers such as polyacrylamide and alginate gels have been used as immobilizing agents.

It is unlikely that a readily available commercial formulation of *Heterorhabditis* spp. will be available for several years, and progress is hampered by the financial risk involved in applying commercially produced steinernematids. Information concerning the economic feasibility of producing heterorhabditids for medium sized pest management programs in Canada is lacking. More specifically, no reports compare the costs of purchasing commercial products, with producing nematodes in-house.

My objectives were to evaluate some of the factors influencing the appropriateness and effectiveness of *H. megidis* H90 as a biological control agent of selected forest insect pests by:

i) comparing the cost of *in vitro* production of *H. megidis* with the cost of purchasing commercial products to meet the needs of a forest nursery;

ii) examining some of the factors that influence the host-parasite relationship, especially the effect of temperature; and

iii) determining the ability of *H. megidis* H90 to parasitize selected insect pests of nurseries, greenhouses, seed orchards and mature forests under laboratory conditions.

# 2.0 GENERAL MATERIALS AND METHODS

## 2.1 Insects

Late instar *G. mellonella,* originally obtained from Simon Fraser University, Burnaby, B.C., were reared for several generations at the Ontario Forest Research Institute, Ministry of Natural Resources, Sault Ste Marie, ON. The larvae were reared according to Dutky *et al.* (1962) on a diet of 100 mL Torula yeast (Lallemand Inc., Montreal, QU), 454 g of Mixed Pablum (H. J. Heinz, Leamington, ON), 200 mL of raw wheat germ, 100 mL of honey, and 125 mL of glycerin.

Larval Douglas-fir cone gall midges were collected from Douglas-fir cones, *Pseudotsuga menziesii* (Mirb.) Franco, in August 1992 and 1993, at the Canadian Forest Products, Ltd. seed orchard, Sechelt, B. C. The cones were immersed in aluminum tubs filled with tap water. Emergent third instar larvae were caught on a fine mesh screen lining the bottom of the tub, and held at 20 °C in the dark up to 7 d until used. Spruce cone maggots were collected in June, 1993, from black spruce cones from a plantation at Lakehead University, Thunder Bay, ON. Third instar larvae were captured with a fine paintbrush as they emerged from cones soaking in water and used within 1 wk of collection. Sixth instar eastern spruce budworm larvae were obtained from the Forest Pest Management Institute, Canadian Forest Service, (FPMI), Sault Ste. Marie, ON, and used immediately. Fungus gnat larvae were obtained from infested acorns of red oak, *Quercus rubra* L., growing in greenhouses at FPMI. The acorns were pried open and

the larvae were retrieved with a fine paint brush and used immediately. Sixty larval root weevils were obtained from Compartment A15 at the Ontario Ministry of Natural Resources bareroot nursery, St. Williams, ON. The larvae were shipped in nursery soil and stored up to 7 d at 20 °C until used.

## 2.2 Nematodes

*Heterorhabditidis megidis* H90, isolated from the Okanagan Valley and the Thompson Canyon areas of B. C. (Mracek and Webster, 1993) and maintained in culture, were obtained from J. M. Webster, Simon Fraser University. Subsequent generations were cultured in late instar larvae of *G. mellonella*, using a modification of the method described by Dutky *et al.*(1964). Each larva was challenged with about 20 IJs of *H. megidis* H90 in 9-cm diam Petri plates (10 larvae per plate) lined with one piece of Whatman No. 3 filter paper moistened with 1 mL sterile distilled water. After 2 d, the cadavers were transferred to White traps (1927) and cultured at *ca.* 20 °C in the laboratory with a 12:12 L:D photo regime. Infective juveniles emerged in 7-10 d, and were maintained up to 7 d at *ca.* 20 °C in water in the traps until used.

*Steinernema carpocapsae* All strain were obtained from Biosys (Palo Alto, CA.), as Biosafe®, and stored at 16 °C. When nematodes were required, a small section of the screen containing the nematodes was cut off. The nematodes were released in water, in compliance with the label instructions.

## 2.3 Inoculation Procedure

Inoculum for experiments were prepared with *H. megidis* H90 recovered from the White traps by pipetting several mLs of the aqueous suspension of IJ's into a sterile 250 mL flask containing 150 mL sterile, distilled water. The flask was shaken manually for 15 s, and a preliminary estimate was made of the number of IJ's in 10 aliquots of 0.01 mL each. A final suspension was obtained by conducting a series of estimates as above and adding sterile, distilled water until the suspension was 200 to 300 IJ's per mL. Once obtained, the suspension inocula was calculated by counting the IJ's in 100 drops (0.01 mL each) of the suspension. Approximately 1 mL of suspension was added to 9 cm diam Petri plates lined with one piece of Whatman No. 3 Filter paper. Sterile, distilled water was used to bring the final volume of the suspension in each treatment plate to 2.0 mL. Control plates were prepared by placing one piece of Whatman No. 3 Filter paper in a 9 cm diam Petri plate to which was added 2 mL sterile, distilled water. One insect was added to each plate. The plates were sealed with Parafilm® and incubated in darkness at temperatures and durations specific to each experiment (see following sections).

*Steinernema carpocapsae* All strain inocula were prepared and quantified as above.

## 2.4 Statistical Methods

Data, unless otherwise stated, are shown as means ± standard errors. Means were compared by Studentized *t*-tests (SAS, 1987). $LT_{50}$'s were determined using probit analysis. Median lethal time and the standard deviation was calculated using a method based on class marks (Hewlett and Plackett, 1979). The number of larvae that died in the intervals (hours) between successive observations (classes) was recorded. Class marks are the midpoints between consecutive classes. The calculations follow the routine of an ordinary grouped distribution with estimates of mean and variance calculated from S*fx*, S*fx*2, and N=s*f* (Hewlett and Plackett, 1979). Comparison of invasion rates of *H. megidis* and *S. carpocapsae* at various times were analyzed using a contingency table analysis and $X^2$ test (Zar, 1984).

# 3.0 SOME DETERMINANTS OF PATHOGENICITY OF *HETERORHABDITIS MEGIDIS*.

## 3.1 Comparison of the rate of invasion of *Heterorhabditis megidis* with that of *Steinernema carpocapsae*

### 3.1.1 Experiment

Four hundred 9-cm diam Petri plates were prepared with nematode inoculum as described in section 2.3. Treatments were: 300 IJ's of *H. megidis* per plate (100 plates), 300 IJ's of *S. carpocapsae* per plate (100 plates), and sterile, distilled water controls (200 plates). One wax moth larva was placed in each plate which was then sealed and incubated at 24 °C. After 1, 2, 4, 6 and 8 h, the larvae were removed, rinsed vigorously for 30 s under running, distilled water, and placed into sterile 9 cm diam Petri plates lined with one piece of moist Whatman No. 3 filter paper; these plates were also sealed with Parafilm and incubated at 24 °C. After 7 d, the larvae were dissected and the numbers infected with nematodes recorded.

### 3.1.2 Results

The *G. mellonella* larvae exposed to *H. megidis* for either 1 or 2 h were all healthy and beginning to pupate when examined 7 d following exposure (Table 4). After 8 h exposure, only 50 % succumbed to infection. In contrast, *S. carpocapsae* caused 35% infection after only 1 h exposure and 75 % after 6 h. Infection by *S. carpocapsae* after

Table 4. Infection rate of *Galleria mellonella* larvae challenged with either *Heterorhabditis megidis* H90 or *Steinernema carpocapsae* All strain at 24 $^{0}$C for five exposure durations.

| Exposure | Percent of larvae infected (N=20 larvae per duration) | | |
|---|---|---|---|
| duration (h) | *Heterorhabditis megidis* | *Steinernema carpocapsae* | $X^2P$ |
| 1 | 0 | 35 | < 0.01 |
| 2 | 0 | 40 | < 0.005 |
| 4 | 25 | 50 | > 0.05 |
| 6 | 20 | 75 | < 0.005 |
| 8 | 50 | 70 | > 0.05 |

all exposure durations was significantly greater than by *H. megidis* (Table 4). There was no nematode infection in either set of controls.

## 3.2 Effect of temperature on the ability of *Heterorhabditis megidis* H90 to penetrate *Galleria mellonella* larvae and to develop into females

### 3.2.1 Experiment

Two hundred 9-cm diam Petri plates were prepared as described in section 2.3. Treatments were 300 IJ's of *H. megidis* per plate (100 plates) and sterile, distilled water controls (100 plates). The plates were sealed , and 20 treated and 20 control plates were placed in each of five growth chambers at 8, 12, 16, 20, and 24 °C. After 24 h, one late instar *G. mellonella* larva was added to each plate, which was resealed and reincubated at its previous temperature in darkness. After 96 h, larvae were examined for the presence of red pigmentation, indicative of the presence of *P. luminescens*, and dissected to determine presence and developmental stage of nematodes.

### 3.2.2 Results

One hundred percent mortality was realized at 16, 20, and 24 °C, but only at 24 °C were all the cadavers red (Table 5). No mortality occurred at 8 or 12 °C. IJ's were present in all plates from 16-24° C. Nematode reproduction, as evidenced by the presence of females, occurred in all larvae held at 20 and 24 °C, but at 16 °C, only 15 % of the test larvae contained females. Among the controls, 7% of the larvae died, but none

Table 5. Effect of temperature on the ability of *Heterorhabditis megidis* H90 to infect late instar larvae of *Galleria mellonella* and develop into females during 96 h in the laboratory. (N=20 larvae per temperature)

| Temperature ($^0$C) | % of larvae infected with nematodes | % dead-larvae | % red larvae | % of larvae containing female nematodes |
|---|---|---|---|---|
| 8 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 |
| 16 | 100 | 100 | 15 | 15 |
| 20 | 100 | 100 | 80 | 100 |
| 24 | 100 | 100 | 100 | 100 |

of these deaths was attributable to nematodes.

## 3.3 Effect of temperature on the number of large female *Heterorhabditis megidis* produced per *Galleria mellonella*.

### 3.3.1 Experiment

One hundred and twenty Petri plates were prepared as described in section 2.3. Sixty were treated with 300 IJ's of *H. megidis* and 60 with sterile distilled water controls. Sixty plates (30 treated with nematodes and 30 control plates) were incubated at 16 °C, and 60 plates were incubated at 24 °C. After 6 h, one *G. mellonella* larva was added to each plate. The plates were resealed, and incubated for a further 32 h, enough time to insure penetration of larvae by the nematodes. Each larva was then rinsed vigorously for 30 s under running distilled water, and placed into a clean Petri plate containing one piece of Whatman No. 3 filter paper moistened with 2.0 mL of sterile distilled water. The plates were resealed, and incubated at 24 °C for 7 days, when the female nematodes were counted.

### 3.3.2 Results

Significantly more nematodes entered the larvae at 24 °C than at 16 °C ($t=6.19$, d.f.=29, $P < 0.001$). From the 30 larvae held 7 days at 24 °C, and the 30 held 7 days at 16 °C, 275 (mean = $9.17 \pm 1.23$ per larva) and 37 (mean = of $1.23 \pm 0.35$ per larva) large female nematodes, respectively, were obtained.

## 3.4 The effect of temperature on the invasion rate of *Heterorhabditis megidis H90* and the release and multiplication rate of *Photorhabdus luminescens* in the hemolymph of *Galleria mellonella*

### 3.4.1 Experiment

Petri plates (160) were prepared as described in section 2.3; 120 plates received 300 IJ *H. megidis* and the 40 remaining plates were inoculated with only sterile distilled water and served as nematode-free controls. After 24 h at 16 $^0$C, one *G. mellonella* larva ( mean weight = 192 ± 2.28 mg; $X_i$=100) was placed into each Petri plate. At approximately 4 h intervals, 10 plates were removed from the growth chamber for examination. By removing a proleg from each larva, a drop of hemolymph was obtained, placed on a hemocytometer, and examined under phase contrast microscopy to determine the number of bacteria. Subsequently, each larva was dissected and the number of IJ's that had entered into the body cavity was recorded. Once bacterial proliferation had begun, collected hemolymph was diluted with phosphate buffered saline[2] to facilitate counting. The experiment was repeated at 24 °C using 160 larvae (mean weight = 226 ± 4.20 mg), with treatments as above.

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[2]Phosphate Buffered Saline (G.B. Dunphy, pers. comm., McGill University, Montreal, QU.): containing 8 g NaCl, 200 mg KCl, 1150 mg $Na_2HPO_4$, and 200 mg $KH_2PO_4$, per liter of distilled water, initial pH 6.2-6.5.

### 3.4.2 Results

*P. luminescens* was first detected in the haemolymph after 32 h of exposure to the nematodes at both 16 and 24 °C (Table 6). At that time, at both 16 and 24 °C, the bacteria multiplied rapidly. At 16 °C, for each duration from 14 h on, all 10 larvae contained IJ's. Such complete infection was not achieved at 24 °C. The mean number of IJ's per larva at 16 °C increased to a maximum of 3.6 at 42 h, but never exceeded 1.0 at 24 °C.

## 3.5. Effect of temperature on $LT_{50}$s of *Heterorhabditis megidis* H90 and *Steinernema carpocapsae* All Strain on *Galleria mellonella*

### 3.5.1 Experiment

Experiments were conducted at 8, 12, 16, 20, and 24 °C for both nematodes. In each experiment, 100 Petri plates were prepared as described in 2.3; 50 plates received an aqueous suspension of 300 IJ's of the nematode species being tested, and the remaining 50 plates received 2 mL of sterile, distilled water and served as controls. The plates were sealed with Parafilm® and placed in growth chambers for at least 8 h prior to the experiment to allow the nematodes to acclimate to the required temperature. One *G. mellonella* larva was then added to each plate which was resealed and returned to the chamber. The larvae were checked at 4 h intervals or at least three times per day for the first 4 days, and about once a day for the following 29 days, or until death of the larva. Dead larvae were dissected and examined for IJ's and female heterorhabditids. The $LT_{50}$'s were calculated using a class marks method (Hewlett and Plackett, 1979).

Table 6. Comparison of the rate of invasion of *Galleria mellonella* larvae in Petri plates at 16 and 24 °C by *Heterorhabditis megidis* H90, and the corresponding multiplication of *Photorhabdus luminescens* in the insect haemolymph. N= 10 per exposure duration. No observations made at 26 and 30 h at 16 °C or 66 h at 24 °C.

| Time | % of larvae with bacteria | | No. of bacteria per larva ($\bar{x}$ ± SE x 1000) | | % of larvae infected with nematodes | | No. of IJ's per larva ($\bar{x}$ +SE) | |
|---|---|---|---|---|---|---|---|---|
| (h) | 16 °C | 24 °C | 16 °C | 24 °C | 16 °C | 24 °C | 16 °C | 24 °C |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 100 | 10 | 2.4 ± 0.67 | 0.1±0.1 |
| 18 | 0 | 0 | 0 | 0 | 100 | 0 | 2.3 ± 0.50 | 0 |
| 22 | 0 | 0 | 0 | 0 | 100 | 30 | 2.7 ± 0.37 | 0.4±0.22 |
| 26 | --[1] | 0 | -- | 0 | - | 50 | -- | 0.5±0.17 |
| 30 | -- | 0 | -- | 0 | - | 60 | -- | 0.7±0.21 |
| 32 | 30 | 10 | 1.5 ± 0.8 | 0.5 ± 0.5 | 100 | 50 | 2.8 ± 0.83 | 0.9±0.35 |
| 38 | 70 | 50 | 113.5± 28.4 | 106.1± 59.9 | 100 | 60 | 3.6 ± 0.54 | 0.9±0.32 |
| 42 | 90 | 90 | 829.5 ± 180.6 | 9,519.6 ± 8,529.4 | 100 | 90 | 3.5±0.54 | 0.6 ±0.16 |
| 66 | 100 | -- | 3,046.0 ± 622.4 | -- | 100 | -- | 0.9 ±0.10 | -- |

Insect hosts were not available for the times denoted with '--'.

### 3.5.2 Results

The length of time required for *H. megidis* or *S. carpocapsae* to kill *G. mellonella* larvae increased with decreasing temperature (Figs. 1 and 2). All larvae in both control treatments remained alive for 33 d. Although *H. megidis* caused 100% mortality at temperatures of 12 to 24 °C, the exposure time was substantially greater at 12 °C (Fig. 1). For *G. mellonella* larvae challenged with *S. carpocapsae*, complete mortality was achieved quickly and simultaneously at temperatures of 16 to 24 °C (Fig. 2). After 10 days at 12 °C, mortality of *G. mellonella* treated with *S. carpocapsae* was 72%.

At 8 °C, all the larvae treated with either nematode species remained healthy to 10 days. After 33 days, 72% of the larvae in the plates treated with *H. megidis* and held at 8 °C, had died. Three of the 36 dead larvae were red, and although the remaining corpses were black from contaminating bacteria, 18 contained IJ's and none contained females. Although reproduction of *H. megidis* was evident at 12°C, it did not occur at 8°C. After 30 days, 94% of the larvae treated with *S. carpocapsae* and held at 8 °C had died.

For larvae challenged with *H. megidis*, $LT_{50}$ values ranged from 41.50 h $\pm$ 0.38 at 24 °C to 180.72 h $\pm$ 0.15 at 12 °C. For larvae challenged with *S. carpocapsae*, $LT_{50}$ values ranged from 43.80 $\pm$ 1.16 at 24 °C to 142 $\pm$ 0.18 at 12 °C (Table 7). Regression analysis obtained by plotting the $LT_{50}$ at 12, 16, 20, and 24 °C for *H megidis* and *S carpocapsae* demonstrated that both nematode species had higher $LT_{50}$ values at lower than at higher temperatures, but that *S. carpocapsae* maintained its infectivity at low temperatures better than *H. megidis* (Table 7).

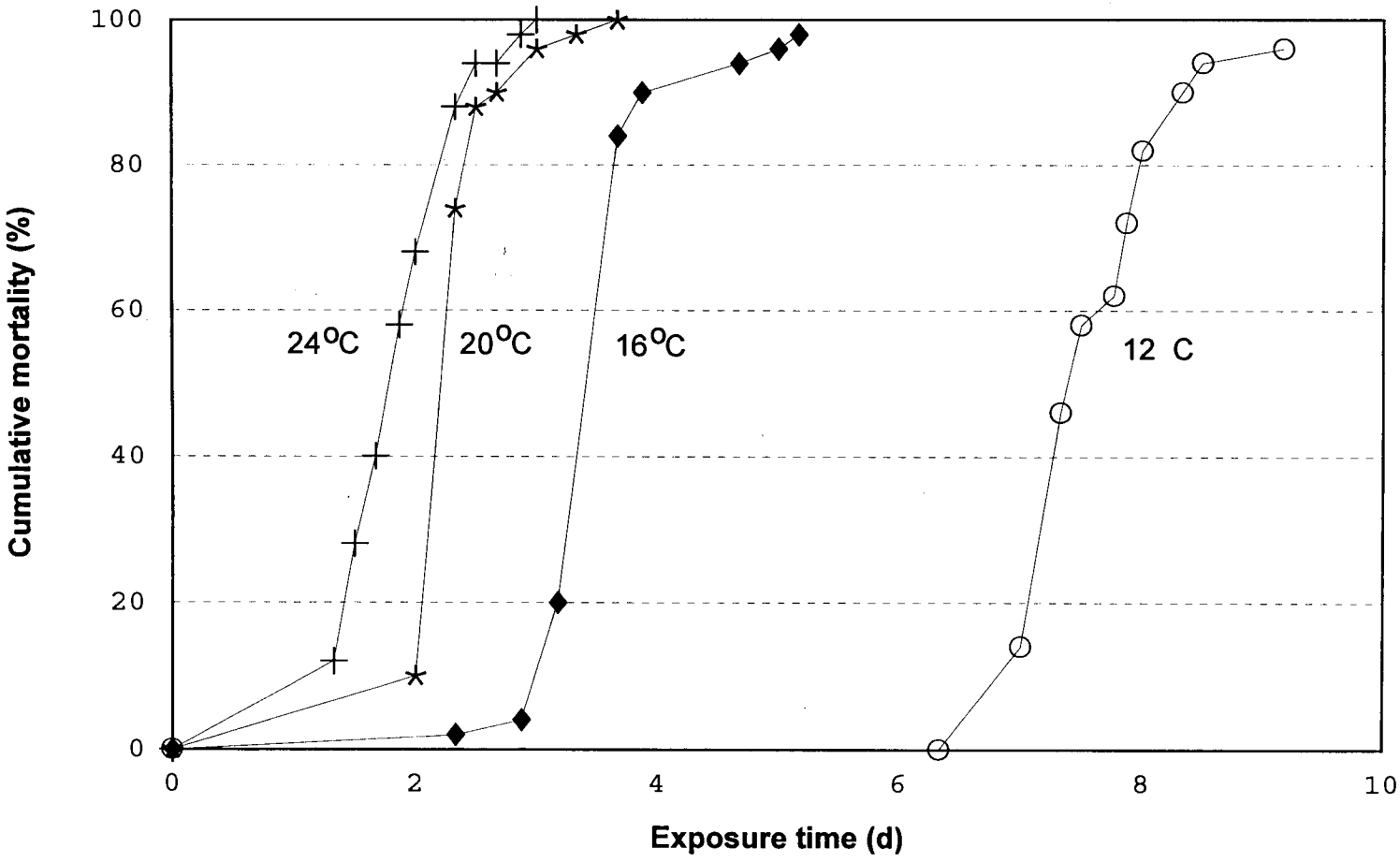


Fig. 1. Cumulative percentage mortality of *Galleria mellonella* larvae challenged in Petri plates with *Heterorhabditis megidis* H90 under four temperature regimes ( N=50 larvae per temperature).

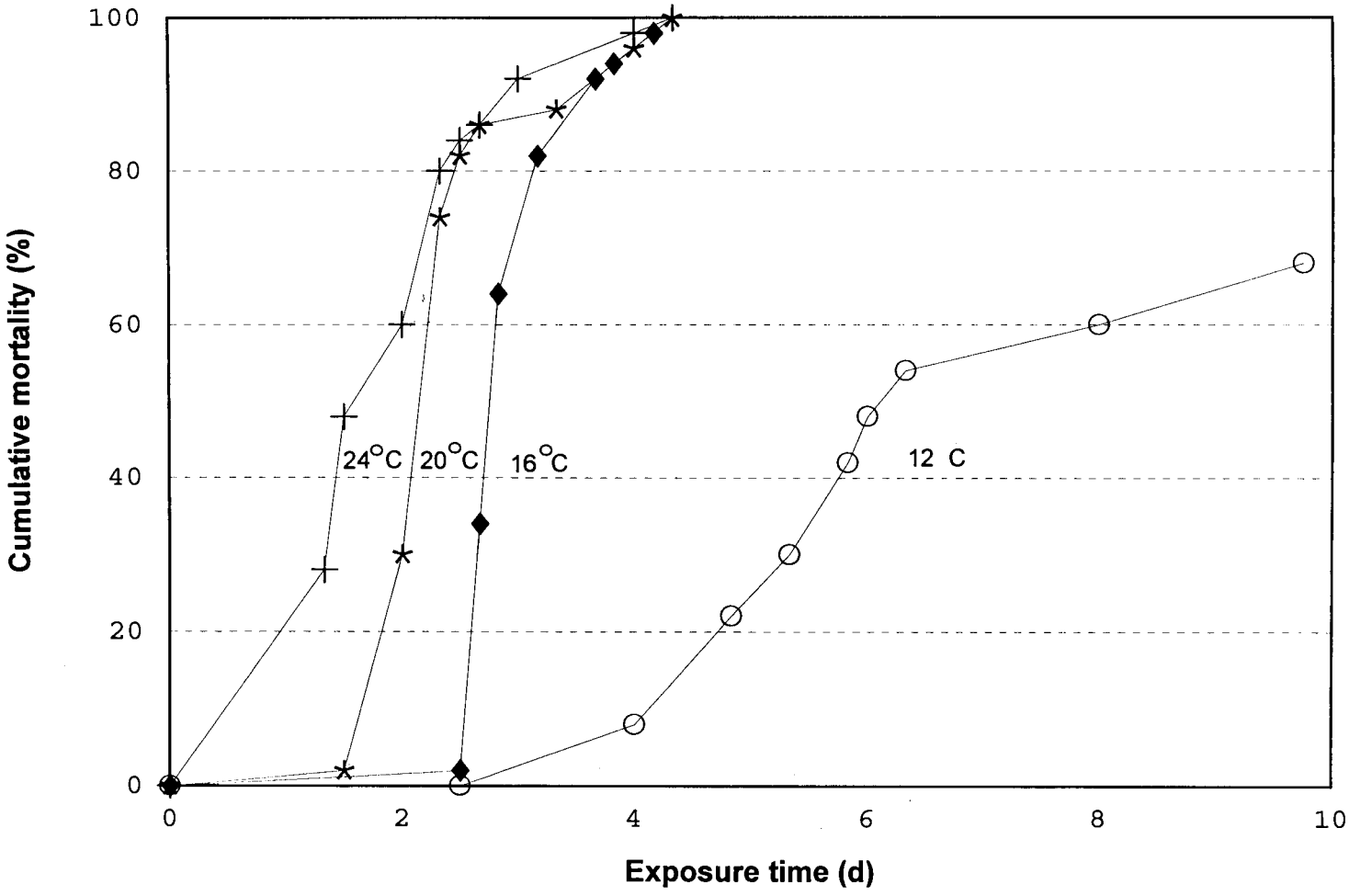


Fig. 2. Cumulative percentage mortality of *Galleria mellonella* larvae challenged in Petri plates with *Steinernema carpocapsae* All strain under four temperature regimes (N=50 larvae per temperature).

Table 7. $LT_{50}$ values for late instar *Galleria mellonella* larvae challenged with infective juveniles of *Heterorhabditis megidis* H90 or *Steinernema carpocapsae* All strain. N=50 *Galleria mellonella* larvae per treatment.

| Temperature | $LT_{50}$ (h) ± SE | |
|---|---|---|
| oC | *H. megidis* | *S. carpocapsae* |
| 12 | 180.72 ± 0.15 | 142.0 ± 0.18 |
| 16 | 85.51 ± 0.16 | 64.74 ± 0.17 |
| 20 | 55.59 ± 0.14 | 63.83 ± 0.18 |
| 24 | 41.50 ± 0.38 | 43.80 ± 0.16 |

## 3.6 Search Behavior of *Heterorhabditis megidis*

### 3.6.1 Experiment

Experiments designed by Lewis *et al.* (1992a) were modified to determine whether *H. megidis* responds to airborne and chemical cues. Petri plates (9 cm diam) were marked with four equal size quadrants and concentric rings of 33, 53 and 73 mm diam. Each plate was filled with a thin layer of 2% water agar and allowed to cool. Using a hot, iron, dissecting probe, a 5 mm diam hole was made in the side of each Petri plate above the agar surface to accommodate a 5 mL plastic pipette tip containing three live, last instar *G. mellonella* larvae (Fig. 3). The tip of the pipette was shortened with a hot scalpel so that the length and tip diam were 7 and 3 cm, respectively. Control treatments were empty pipette tips. The pipette tips were put into place 1 h prior to an experiment to allow a volatile gradient to form.

An aqueous suspension of nematodes was prepared to a concentration of 100 IJ's per mL as described in section 2.3.. A 13 mm diam disk of Whatman No. 1 filter paper was placed onto a 13 mm diam vacuum cup and 2 mL of sterile distilled water was run through the filter to wet the paper and secure it to the bottom of the filter. A 1.0 mL aliquot of the nematode suspension was then passed through the vacuum. The filter paper was removed and placed in the centre of the Petri plate through a hole created with a hot sterilized dissecting probe in the top of the Petri plate. The ports on the top and sides of the plate, and the entire sides were sealed with Parafilm. Nematodes in the quadrants closest to and furthest from the *G. mellonella* larvae were counted after 5, 10, 15, and 20

min. Mean distance (mm) travelled in response to the treatment was calculated using the equation:

$$\bar{x} = [(10 \times A_1) + (20 \times A_2) + (30 \times A_3)] - [(10 \times B_1) + (20 \times B_2) + (30 \times B_3)] / 100$$

where the numbers of nematodes in the first, second and third arcs of the proximal and distal quadrants are represented by $A_1$, $A_2$, and $A_3$, and $B_1$, $B_2$, and $B_3$, respectively (Fig. 3). Experimental and control treatments were both replicated 10 times.

### 3.6.2 Results

IJ's of *H. megidis* were strongly attracted to live *G. mellonella* larvae, and after 20 min, had moved $2.71 \pm 0.37$ mm toward the host stimulus (Fig. 4). In the control treatments, there was an unexplained, slight movement by IJ's in the direction of the empty pipette. A significant difference between movements of nematodes in control and experimental treatments arose after 20 min ( $P < 0.006$).

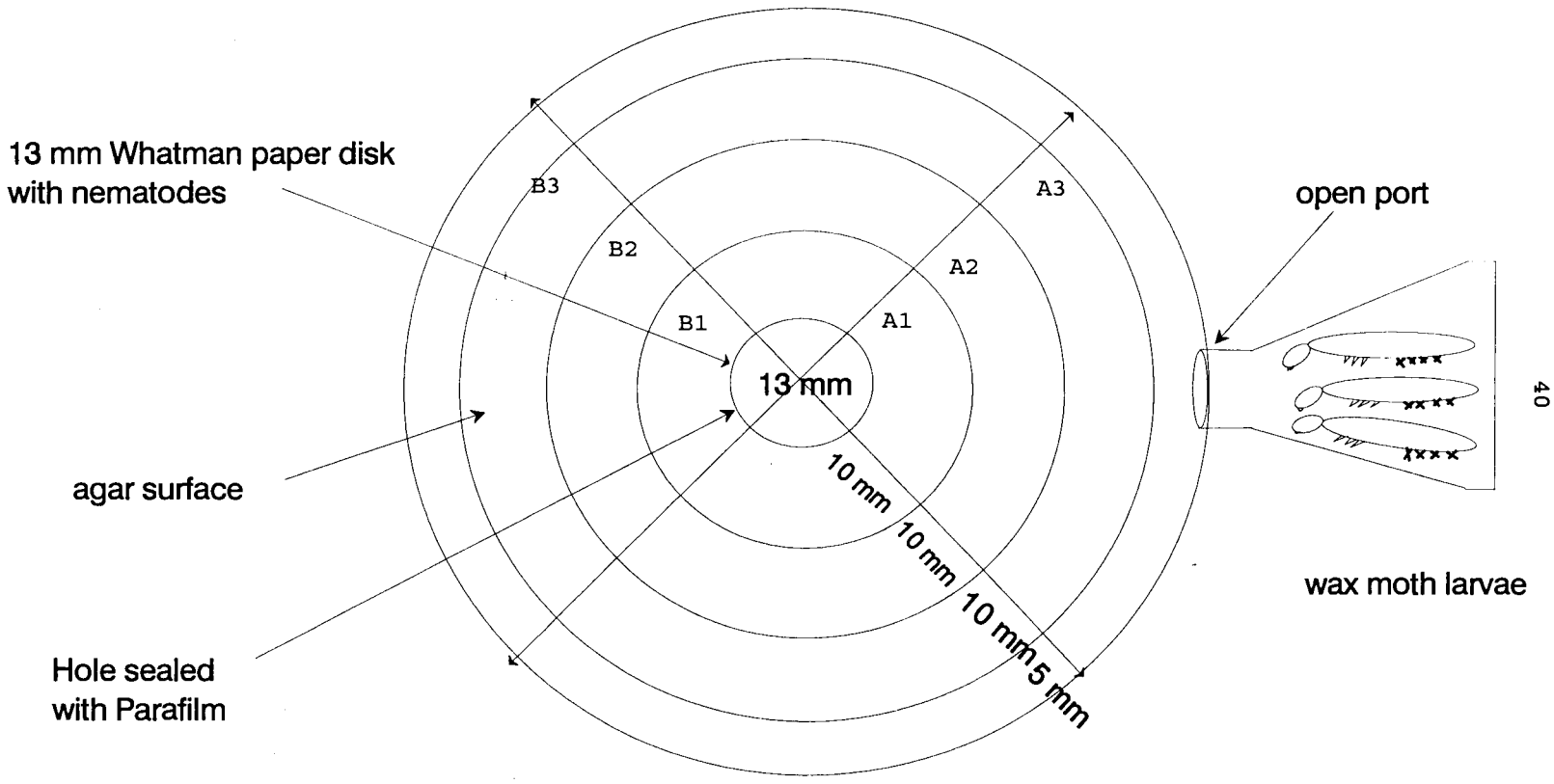


Fig. 3. Petri plate arena divided into quarters and concentric zones used to evaluate the movement of IJs of *Heterorhabditis megidis* H90 in response to chemical stimuli produced by living *Galleria mellonella* larvae.

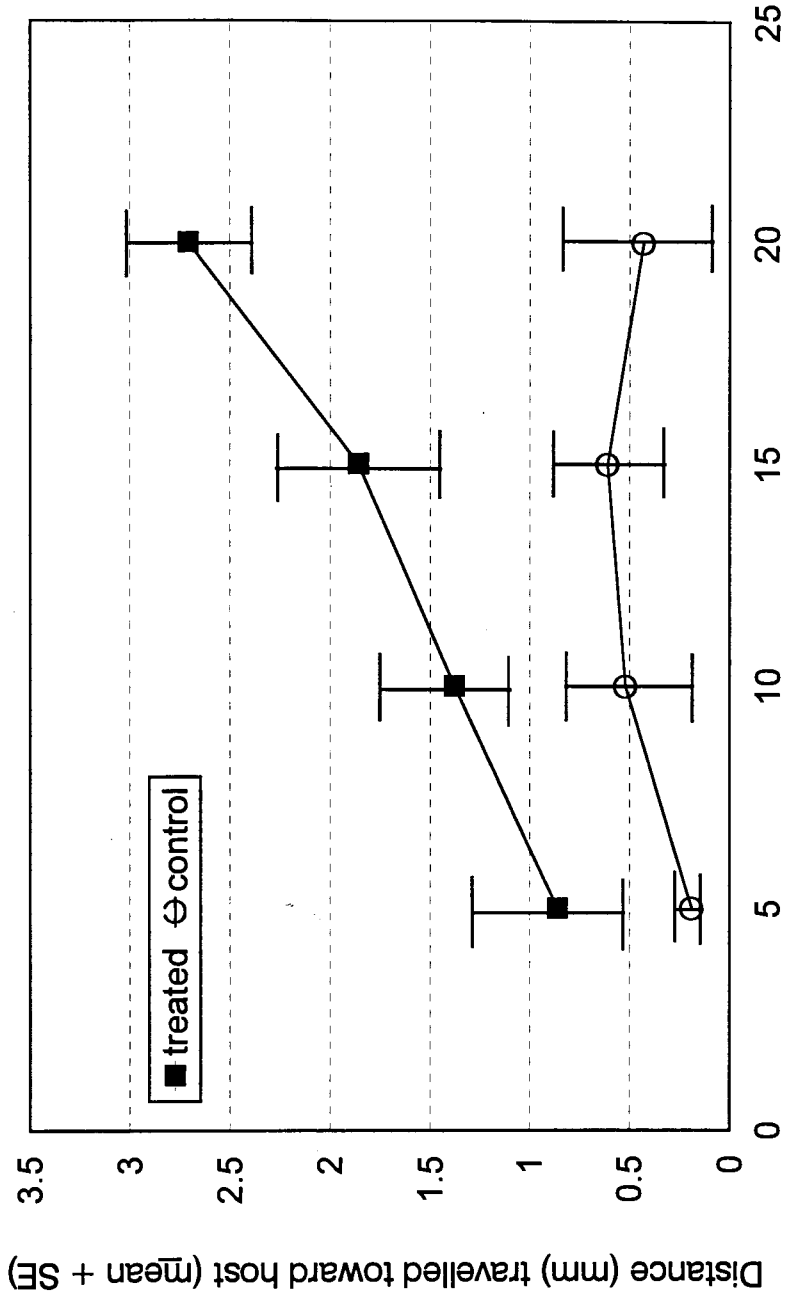


Fig. 4. Distance travelled by IJ *Heterorhabditis megidis* H90 toward *Galleria mellonella* larvae in a Petri dish arena over a 20 min duration.

# 4.0 POTENTIAL OF *HETERORHABDITIS MEGIDIS* H90 AS A BIOCONTROL AGENT FOR FOREST INSECT PESTS

## 4.1 Douglas-fir Cone Gall Midge, *Contarinia oregonensis*

### 4.1.1 Experiment

Petri plates (308) were prepared as described in section 2.3. Two hundred and twenty eight third instar larvae of the midge and 80 late instar *G. mellonella* larvae were used in the experiment. Treatment plates (159) received 1.0 mL inocula of an aqueous solution containing 300 IJs *H. megidis* and 1.0 ml sterile distilled water, and control plates (149) received 2 mL of sterile, distilled water. Midge larvae (119), and 40 late instar *G. mellonella* larvae were placed individually into experimental plates. To ensure insect-nematode contact, a 9 cm diam filter paper was placed on top of each midge to prevent the larvae from "jumping" up and adhering to the plate lid. A 1.0 mL drop of sterile, distilled water was added to the top filter paper to enable free movement of the nematodes over its surface. The remaining midge and *G. mellonella* larvae were placed in control plates. All plates were sealed with Parafilm®, and divided into two equal groups, half of which were incubated in the dark, at 24 °C, the other half were incubated in the dark at 20°C. After 5 d, each larva was dissected and examined for nematodes.

### 4.1.2 Results

Less than a third of the treated *C. oregonensis* larvae died as a result of nematode infection compared to 100% mortality of treated *G. mellonella* larvae (Table 8) There

Table 8. Percentage of *Contarinia oregonensis* and *Galleria mellonella* larvae living, dead, and infected with nematodes after exposure to 300 IJ *Heterorhabditis megidis* H90 per larva over 5 d in Petri plates at 20 and 24°C.

| Test species | Temp (°C) | Treatment | N | % living | % Dead | |
|---|---|---|---|---|---|---|
| | | | | | With nematodes | Without nematodes |
| *C. oregonensis* | 20 | *H. megidis* | 60 | 83 | 15 | 2 |
| | | Control | 50 | 98 | 0 | 2 |
| | 24 | *H. megidis* | 59 | 65 | 27 | 8 |
| | | Control | 59 | 93 | 0 | 7 |
| *G. mellonella* | 20 | *H. megidis* | 20 | 0 | 100 | 0 |
| | | Control | 20 | 100 | 0 | 0 |
| | 24 | *H. megidis* | 20 | 0 | 100 | 0 |
| | | Control | 20 | 100 | 0 | 0 |

was no difference in percent mortality of *C. oregonensis* between temperatures ($X^2$ = 2.53, d.f. = 1, $P > 0.05$). No mortality in control treatments was attributable to nematode infection.

## 4.2 Spruce cone maggot, *Strobilomyia appalachensis*

### 4.2.1 Experiment

Two aqueous suspensions of IJ's of *H. megidis* H90 were prepared, one with 500 - 2000 IJ's per mL, and the other with 2000-8000 IJ's per mL. Ninety six 6-cm diam Petri plates containing a 2:1 peat : vermiculite mixture about 15 mm deep were prepared and moistened with 5 mL sterile distilled water. Thirty six of the plates received 1.0 mL of nematode suspension containing 2000-8000 IJ *H. megidis*, 36 plates received 1.0 mL of nematode suspension containing 500- 2000 IJ *H. megidis*, and 24 plates received 2 mL sterile, distilled water and served as controls. One *S. appalachensis* larva was placed in half of the treated and control plates, and one late instar *G. mellonella* larva was placed into the remaining plates. The plates were sealed with Parafilm® and incubated at 20 °C for 5 whereupon the larvae were dissected and examined for nematodes.

To assess the ability of *H. megidis H90* to penetrate *S. appalachensis* pupae, a trial was conducted using 70 pupae, each placed in a 6 cm diam Petri plate lined with one piece of Whatman No. 3 filter paper. Exposure to *H. megidis*, incubation, and examination were as in the first experiment.

4.2.2 Results

Mortality of *S. appalachensis* larvae was significantly different ( $X^2 = 5.89$, d.f. = 1, $P < 0.05$) for nematode inocula of 2000-8000 and 500-2000 IJ *H. megidis* (Table 9). Mortality of *S. appalachensis* was highest at the low dose; the opposite trend occurred for *G. mellonella*. No *G. mellonella* or *S. appalachensis* larvae died in the controls. In the second experiment 7% of *S. appalachensis* pupae, and all of the *G. mellonella* larvae were infected by nematodes (Table 9).

## 4.3 Mortality of Eastern Spruce Budworm, *Choristoneura fumiferana*, Root weevils, *Otiorhynchus ovatus*, and Fungus gnats, *Bradysia* sp.

4.3.1 Experiment

In separate experiments for each insect species, Petri plates were prepared as described in 2.3. In the first experiment, 200 Petri plates were prepared; 100 plates received 1.0 mL of an aqueous suspension containing 300 IJ *H. megidis* and 1.0 mL of sterile distilled water, and 100 plates received 2.0 mL of sterile, distilled water. One late instar *C. fumiferana* larva was placed in each of 50 treated and 50 control plates, and one late instar *G. mellonella* larva was placed in each of 50 treated and 50 control plates. In the second experiment, 120 Petri plates were prepared; 60 plates received 1.0 mL of an aqueous suspension containing 300 IJ *H. megidis* and 1.0 mL of sterile distilled water, and 60 plates received 2.0 mL of sterile distilled water. One *O. ovatus* larva was placed

Table 9. Percentage of *Strobilomyia appalachensis*, larvae and pupae, and *Galleria mellonella* larvae living, dead, and infected with nematodes after exposure to *Heterorhabditis megidis* H90 over 5 days in Petri plates at 20 °C.

| Experiment No | Test species and stage | Treatment | N | Percent Dead | |
|---|---|---|---|---|---|
| | | | | With nematodes | Without nematodes |
| 1 | *Strobilomyia appalachensis* larvae | 2000-8000 IJ *H. megidis* | 18 | 17 | 0 |
| | | 500- 2000 IJ *H. megidis* | 18 | 56 | 0 |
| | | Control | 12 | 0 | 0 |
| | *Galleria mellonella* larvae | 2000-8000 IJ *H. megidis* | 18 | 44 | 1 |
| | | 500-2000 IJ *H. megidis* | 18 | 28 | 0 |
| | | Control | 12 | 0 | 0 |
| 2 | *Strobilomyia appalachensis* pupae | < 2000 IJ *H. megidis* | 70 | 7 | 0 |
| | *G. mellonella* larvae | < 2000 IJ *H. megidis* | 20 | 100 | 0 |

in each of 30 treated and 30 control plates, and one late instar *G. mellonella* larva was placed in each of 30 treated and 30 control plates. In the third experiment, 185 experimental Petri plates were prepared; 98 plates received 1.0 mL of an aqueous suspension containing 300 IJ *H. megidis* and 1.0 mL of sterile distilled water, and 87 received 2.0 mL of sterile distilled water. One *Bradysia* sp. larva was placed in each of 48 treated and 37 control plates, and one late instar *G. mellonella* larva was placed in each of 50 treated and 50 control plates. All plates were sealed and incubated in the dark at 16 °C. After 5 days, all larvae were dissected and examined for nematodes.

### 4.3.2 Results

In the first experiment, total mortality was achieved 5 d after nematode treatment for both *C. fumiferana* and *G. mellonella* (Table 10). No mortality occurred in the control treatments. In the second experiment, 30 % mortality of root weevil larvae was caused by the nematodes; 13% of the untreated larvae died during 5 d, but not from nematode infection (Table 10). All treated *G. mellonella* larvae died, but no mortality occurred in the *G. mellonella* control larvae. In the third experiment, 91 % of the treated fungus gnat larvae died, and all but one of these deaths were attributed to nematode infection (Table 10). During 5 d , 40% percent of the control *Bradysia* sp. died, but not from nematode infection. All treated *G. mellonella* larvae and none of the controls died.

Table 10. Percentage of larval *Choristoneura fumiferana*, *Otiorhynchus ovatus*, *Bradysia* spp., and *Galleria mellonella,* larvae living, dead, and infected with nematodes after exposure to 300 IJ *Heterorhabditis megidis* H90 per larvae over 5 days in Petri plates at 24 °C.

| | | | | % Dead | |
|---|---|---|---|---|---|
| Experiment number | Test species | Treatment | N | With nematodes | Without nematodes |
| 1 | *C. fumiferana* | *H. megidis* | 50 | 100 | 0 |
| | | control | 50 | 0 | 0 |
| | *G. mellonella* | *H. megidis* | 50 | 100 | 0 |
| | | control | 50 | 0 | 0 |
| 2 | *O. ovatus* | *H. megidis* | 30 | 30 | 0 |
| | | control | 30 | 0 | 13 |
| | *G. mellonella* | *H. megidis* | 30 | 100 | 0 |
| | | control | 30 | 0 | 0 |
| 3 | *Bradysia* sp. | *H. megidis* | 48 | 91 | 1 |
| | | control | 37 | 0 | 40 |
| | *G. mellonella* | *H. megidis* | 50 | 100 | 0 |
| | | control | 50 | 0 | 0 |

## 4.4 Control of *Bradysia* spp. in a Greenhouse

### 4.4.1 Experiment

Six, 52 x 26 x 3 cm trays containing red oak seedlings, *Quercus rubra* L., growing in potting mixture which was heavily infested with fungus gnat larvae and adults were placed in a greenhouse cubicle (about 3 x 5 x 4 m high). Another cubicle contained six similar control trays of red oak seedlings. The trays were placed on benches 0.6 m above the ground, watered daily to saturation, and kept under natural lighting conditions. Greenhouse temperatures in May ranged from 20 to 25 °C. The number of adult fungus gnats was estimated and monitored using sticky traps (Rutherford *et al.*, 1985) made from white plastic, plant-labels coated with "STP" motor oil additive. Two traps per tray were placed in opposite corners of each tray. The first traps were placed on May 4 and nematode suspensions of 150,000 IJ's per $m^2$ were applied on the 10th, 16th, and 20th of May. Adult fungus gnats caught on the traps were counted May 10, 16, 20, and June 3.

### 4.4.2 Results

The numbers of fungus gnats caught over experimental and control trays were not statistically different ( $t = 1.3762$, d.f. = 22, $P > 0.18$) before the first application (Fig. 7). By 6 d after the first application, the numbers were significantly lower ($t = 4.25$, d.f. = 22, $P < 0.003$), and the population in the cubicle containing the treated trays remained substantially lower than that in the control cubicle for the duration of the trial.

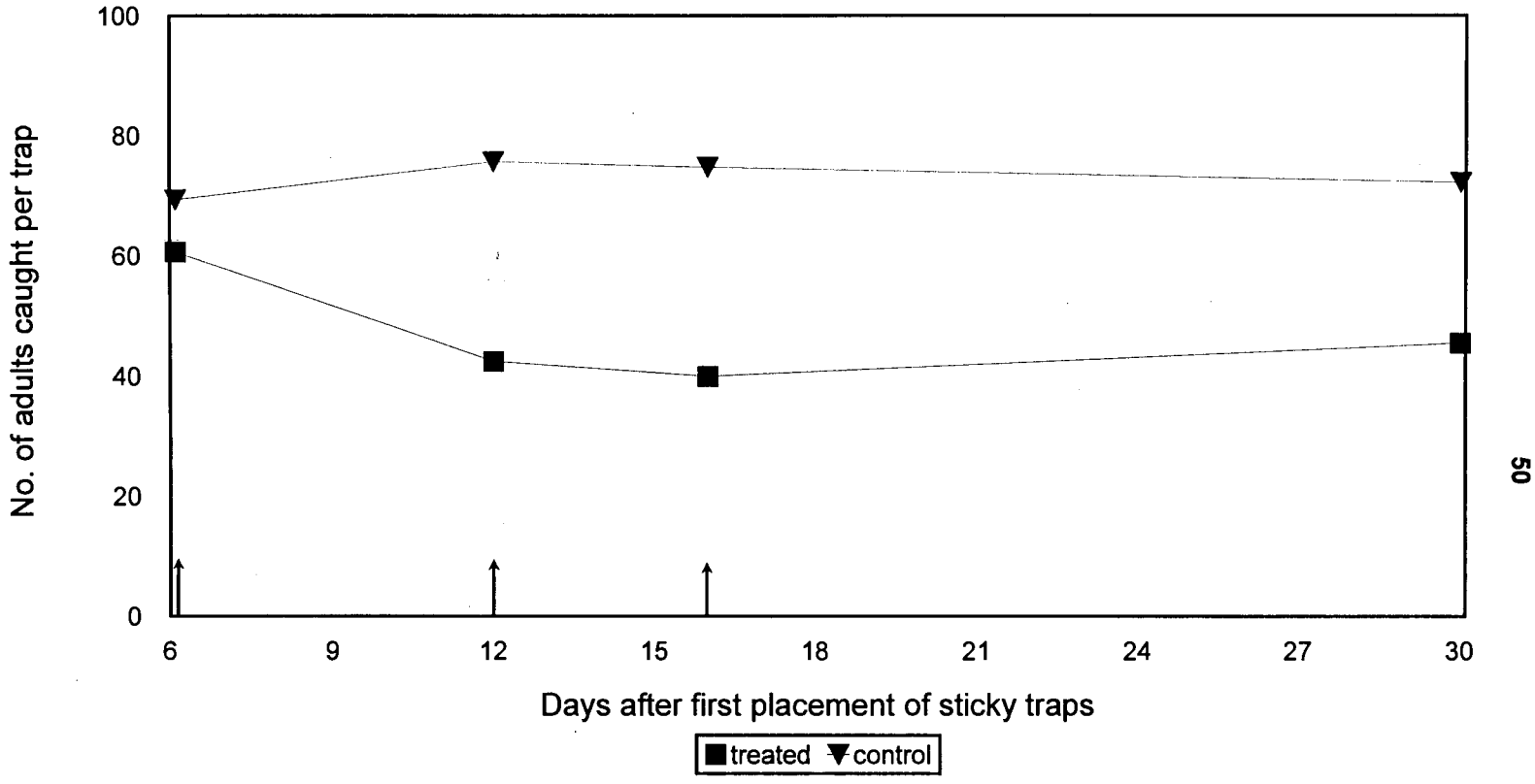


Figure 6. The effect of three successive applications (arrows) of *Heterorhabditis megidis* H90 to trays of red oak seedlings (*Quercus rubra*) infested with *Bradysia* spp. as measured by the number of adult *Bradysia* spp. caught on sticky traps over a 1 month period during and following treatment.

## 5.0 DISCUSSION

Table 3 indicates that from an economic perspective, the application of commercial nematodes remains substantially more costly than using conventional pesticides. Direct cost, however, must be weighed against the cost of resistance to conventional pesticides, plus environmental and health concerns. Even before cost is considered, the efficacy of nematode formulations and conventional pesticides must be compared.

The cost of treating an entire bareroot nursery (which may exceed 40 ha) with nematodes is prohibitive. However, it may be feasible to use commercially produced nematodes if the application is judicious rather than widespread across entire nursery beds. For example, insect larvae may be most plentiful in the rows adjacent to hedgerows and it may be sufficient to treat only the outer rows. It may also be possible to combine nematodes with chemical insecticides in spray applications since nematodes may survive when applied in combination with many pesticides. Such combinations may allow pest management programs to remain effective with reduced applications of conventional pesticides.

In-house production of heterorhabditids is too labour intensive for use in forest nurseries unless the bag method of Bedding (1984) were to be used. Prior to using the bag method, *in vitro* production in 1 L flasks must be attained. In Ontario, it may be possible to optimize the Bedding method by using fermenters available in government laboratories and improving upon the substrate used to carry the crude food base by using new porous products that have only recently come on the market. Fermentation would

allow nematode production to be a continuous process whereby most of the nematodes could be harvested after several weeks, and the remaining 20% would be left in the fermenter as inoculum to which new food could be added.

The more rapid infection rate of *S. carpocapsae* All strain compared to *H. megidis* H90 (Table 4) supports Glazer's (1992) report that the time needed for 50 % mortality of *G. mellonella* larvae was 0.5-2.0 h and 5.0 -14.11 h for *S. carpocapsae* All strain and *H. bacteriophora,* respectively. In the same study, *Heterorhabditis* sp. (strain IS) caused 50 % mortality of *G. mellonella* larvae in 0.8- 2.0 h, demonstrating the great variability in virulence among strains.

In the temperature-dependent mortality experiment, the observation that only 15% of the larvae killed by *H. megidis* H90 at 16 °C had turned red after 96 h may be explained by slower invasion by the nematode and slower release of the bacteria at 16 °C than at 20 or 24 °C. This experiment did not show whether temperature affects the invasion rate of nematodes, but it does indicate that the bacteria can survive and slowly reproduce at 16 °C.

The relationship between temperature and *Heterorhabditis* spp. infectivity has been explored by Zervos *et al.* (1991) and Wright (1992), but my results demonstrate that low temperatures result in both a slow rate of infection and a slow rate of development of the IJ's to adult females within the cadaver. However, since nematode application in the field usually takes the form of inundative releases, it is of limited relevance whether or not the nematodes reproduce in the field.

The apparently contradictory results in Tables 4 and 5 with regard to infectivity at 16 and 24 °C may be explained as experimental error since different nematode suspensions were used. Considering that bacterial invasion occurred at the same hour at both temperatures, low $LT_{50}$ values at high temperatures may be the result of the bacterial growth rather than the speed of invasion by nematodes.

The $LT_{50}$ values of *H. megidis* at 24 and 20 °C of 42 ± 1 h and 56 ± 1 h, respectively (Table 7), were similar to the values of 37 ± 3 h and 60 ± 3 h found for the *H. heliothidis* NC 1 strain (Dunphy and Webster, 1986). However, the $LT_{50}$ of 85 ± 1 h for *H. megidis* at 16 °C was much lower than the value of 175 ± 5 h found by Dunphy and Webster (1986). This difference may indicate that *H. megidis* is better adapted to cold conditions than *H. heliothidis*.

The higher $LT_{50}$ values for *H. megidis* than for *S. carpocapsae* at 16 and 12 °C (Table 7) indicate that although both nematodes can infect *G. mellonella* larvae at temperatures between 12 and 24 °C, *S. carpocapsae* is more suited than *H. megidis* to killing at the low end of the temperature range. The incomplete mortality of *G. mellonella* larvae challenged with *S. carpocapsae* at 12 °C (Fig. 2) is likely due to experimental error such as growth chamber malfunctions.

The lack of infection by either *H. megidis* or *S. carpocapsae* after 10 d at 8 °C suggests that neither is suitable for biocontrol at temperatures below 12°C. The finding that nematode parasitism eventually occurred at 8 °C, even though development did not ensue, was also reported by Molyneux (1986). Similarly, Zervos *et al.* (1991) found that at 5 and 10 °C, even after 60 d, there was no emergence of IJs of *H heliothidis*

from *G. mellonella* larvae. Wright (1992) reported that at 8 °C, after a 60 d incubation, heterorhabditids failed to emerge from *G. mellonella* larvae.

Temperature influences nematode mobility (Molyneux, 1986), reproduction and development (Zervos *et al.*, 1991; Wright, 1992), successful application as biological control agents (Burman and Pye, 1980) and infectivity (Finney and Bennett, 1984). Steinernematids remain mobile at temperatures as low as 4 °C while the lower threshold of mobility for heterorhabditids is 10 - 14 °C, depending on the strain (Molyneux, 1986). Unlike heterorhabditids, steinernematids reproduce at temperatures as low as 10 °C (Wright, 1992). Finney and Bennett (1984) demonstrated that *C. fumiferana* larvae parasitized by *H. heliothidis* died more slowly in Petri plates at 17 °C than at 24 °C. My results demonstrate that temperature influences the number of IJ's entering the host insect at any given time, the subsequent growth of the bacteria, and nematode development. They also show that the temperature range over which *H. megidis* H90 is infective differs from that of *S. carpocapsae* All strain, and suggests that the tests used to evaluate and compare the influence of temperature on *H. megidis* and *S. carpocapsae* could be used to evaluate nematode strains.

The orientation of IJ *H. megidis* to volatiles from live *G. mellonella* larvae (Fig. 4) is similar to that reported by Lewis *et al.*, (1992a) for *S. glaseri.* The latter species moved 6.5 mm toward *G. mellonella* larvae in 20 min, but *S. carpocapsae* showed no net movement during the same period. While *S. carpocapsae* has been labelled an ambusher (Lewis *et al.*, 1992b), *S. glaseri* (Lewis *et al.*, 1992a) and *H. megidis* H90 (Fig. 5) may be categorized as cruisers that seek out their prey. It is becoming apparent

that knowledge of the host-seeking strategy of a nematode is vital in deciding which nematode to use against a specific pest. For example, *S. carpocapsae* may be very successful in the Petri plate environment, especially against a larva like *G. mellonella* which continually cruises the Petri plate, but may fail to find and penetrate a sessile pest larva overwintering in the soil.

To evaluate the pathogenicity of nematodes, researchers have adopted the same useful methods ( $LT_{50}$'s and $LD_{50}$'s) used to evaluate chemical pesticides (Glazer, 1992). Typically, these evaluations report the mortality of insect hosts challenged with IJ's in Petri plates containing moist filter paper. Although these values are useful, they mask other factors that are intricately involved in pathogenesis, namely, the number of bacteria per IJ ( Dunphy *et al.*, 1985), the virulence of the associated bacteria, the length of time required to invade the host (Glazer, 1992) and the subsequent release and multiplication rate of the bacteria, and the host seeking strategy employed by the nematode. Glazer (1992) reported that the $LT_{50}$ varied greatly with the insect species and nematode strain. Rather than basing infectivity ratings on the susceptible *G. mellonella*, it may be advisable to select for a more resistant *G. mellonella*, or include a more resistant insect species as an additional test organism. Although bioassays using *G. mellonella* larvae as test organisms can ascertain nematode viability, *G. mellonella* is so susceptible that the comparative infectivity of different strains may be obscured. In conjunction with techniques designed to measure the other factors involved in pathogenesis, Petri plate tests using both *G. mellonella* and a more resistant species may constitute a meaningful measure of nematode infectivity.

Part of the reason for the low infectivity of *C. oregonensis* by *H. megidis* H90, even under optimal condition in a Petri plate (Table 8), may be attributed to the lack of chemical cues released by the midge larvae. They appear to obtain most of their sustenance by absorption through the cuticle, leave no excreta, and have a nearly functionless digestive system (Felt, 1925). Without the release of chemical stimuli from the larvae excreta, nematodes may not be attracted to the larvae.

The similar mortalities of *S. appalachensis* and *G. mellonella* exposed to *H. megidis* H90 in a peat:vermiculite medium (Table 9) suggests that the nematodes could find and infect the larvae of both species. This finding parallels that of J. Sweeney (pers comm., Forestry Canada, Fredericton, N.B.) who found that spruce cone maggots are susceptible to *Steinernema* spp. Both *C. oregonensis* and *S. appalachensis* have already inflicted damage when they leave the cone (Tripp, 1954). Therefore, if effective nematode applications against C. *oregonensis* or *S. appalachensis* were developed, nematodes would be used in the fall to reduce potentially damaging populations in the following spring. Because larval drop by *S. appalachensis* follows rainfall (Sweeney and Turgeon, 1994), nematode application could also follow rainfall to ensure that the susceptible larvae, rather than the partially resistant pupae (Table 9) are exposed to the nematodes.

In a world list of 1,132 natural enemies of cone and seed insects, > 97% were hymenoptera or diptera; only one listing was attributed to a nematode (Yates, 1989). Mracek and Webster (1993) found that nematodes were most prevalent in ecosystems where human impact had been substantial, but apparently were absent from certain forest

localities where potential host larvae and pupae were commonplace. Bedding *et. al* (1983) found that the least infectious nematodes tested were those that were found in natural association with their hosts. This is contrary to the hypothesis that a nematode strain is likely to be most infectious against its natural host. Thus, nematodes that are not commonly in natural association with cone and seed insects, may be excellent candidates for microbial control. Total mortality of eastern spruce budworm larvae following exposure to *H. megidis* (Table 10) is consistent with the above argument. Earlier studies showed that *H. heliothidis* can kill both early (Finney and Bennett, 1984) and late instars (Finney *et al.*, 1982) of this pest.

For root weevils, the infection rate was not as great as that of *G. mellonella* (Table 10) but might have been increased by adding food material (roots) to the Petri plates (Pye and Burman, 1978). In agreement with my findings that *Otiorhynchus* spp. are susceptible to *H. megidis*, Stimmann *et al.*, (1985) reported 90% mortality in *O. sulcatus* when *H. heliothidis* was applied at a rate of 15,000/pot. The ideal place and time for application of entomopathogenic nematodes against root weevils would be in fields just after a crop has been lifted. At this time, in infested fields, there remains ample tree root segments upon which the larvae feed, and the soil is easy to drench with nematode suspensions. When seedlings are growing, nematode drenches may be ineffective due to the interception of nematodes on foliage. The situation is compounded by raised and well drained nursery soil which may cause the nematodes to be rinsed away.

Larval fungus gnats, proved to be very susceptible to *H. megidis* (Table 10). Larval fungus gnats are very active and it may be that the 40% mortality of the control larvae was

caused by starvation or desiccation. The greenhouse trial demonstrated that a decrease in the adult fungus gnat population occurred following treatment with H. *megidis* H90 (Fig 6). The population crash in both treatments after 30 d is common in fungus gnats in greenhouses and may be caused by disease or environmental conditions. Greater mortality might have occurred if higher applications of nematodes had been used.

Despite the research that has been conducted to date, nematodes are still not used in operational forest pest management programs in Canada, except rarely in greenhouses. If nematodes are to become viable pest control products, they will need to outcompete other microbial insecticides (namely, entomopathogenic viruses, bacteria, protozoa and fungi) for a niche in the market. Nematodes are unique among microbial insecticides in that they are the only organism capable of seeking out hosts that dwell in cryptic habitats, e.g. *C. oregonensis* and *S. appalachensis*. Like *Bacillus thuringiensis* Berliner and some fungi, e.g. *Beauvaria* spp., nematodes have the advantage of being fairly broad in their potential host spectrum. However, methods for enhancing infectivity must be sought before nematodes can be used against such cryptic insects. Just as new research in spray technology and the selection of new strains is being conducted to enhance the use of *B. thuringiensis*, it will be necessary to consider similar types or research for nematodes.

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