COMPATIBILITY OF SELECTED INSECTICIDES WITH TWO STEINERNEMATID NEMATODES

MARCUS GLENN WORTHMAN

COMPATIBILITY OF SELECTED INSECTICIDES WITH TWO STEINERNEMATID NEMATODES

BY

© Marcus Glenn Worthman, B.Sc.(Honours)

A thesis submitted to the School of Graduate

Studies in partial fulfillment of the

requirements for the degree of

Master of Science

Toxicology

Memorial University of Newfoundland

May, 1988

St. John's Newfoundland

ABSTRACT

The combination of insecticides with entomogenous nematodes in integrated pest management protocols could be an effective way of lowering environmental loads of harmful insecticides. The compatibility of two locally isolated steinernematid nematodes, (*Steinernema* sp. Newfoundland strain no. 1 (L1C) and *Steinernema* sp. Newfoundland strain no. 2 (5B)), with the organochlorine insecticides chlordane and methoxychlor, the organophosphates diazinon and malathion, the carbamates carbaryl (Sevin^R) and aminocarb (Matacil^R), as well as the insect growth regulator diflubenzuron (Dimilin^R), was investigated.

Direct exposure of infective juveniles to chlordane, methoxychlor, diazinon and carbaryl at a concentration of 1.0 mg/ml proved toxic. Exposure of nematodes to $\leq$0.1 mg/ml of these three and $\leq$1.0 mg/ml of malathion, carbaryl, Matacil and Dimilin did not cause significant mortality to the juveniles of both nematodes.

Efficacy of infective juveniles to the Greater Wax moth, *Galleria mellonella*, was unaffected, except where nematodes were lethally affected by chlordane, methoxychlor and diazinon at 1.0 mg/ml.

In vitro development of both nematodes was impaired by exposure to all tested insecticides. Toxicity appeared to be related to both direct toxicity towards adult and non-ensheathed nematode larvae as well as towards the associated bacterial symbionts.

The doubling or generation time of the bacterial symbionts, *Xenorhabdus* sp.

L1C and *Xenorhabdus* sp. 5B, was found to be approximately 90 and 117 minutes respectively. Bacteria showed variable sensitivity to the tested insecticides but the carbamates and diazinon were particularly toxic at $\geq$ 0.1 mg/ml.

The juveniles of both nematode strains demonstrated active seeking of hosts by migrating in soil towards *G. mellonella* larvae.

Nematode migration in soil was not impaired by the organochlorine insecticide, chlordane. However the organophosphate diazinon reduced migration of both nematodes at $\geq$0.1 mg/ml.

The data obtained suggest that compatibility of nematodes with insecticides in integrated pest management programs can be attained by lowering insecticide levels or altering timing of insecticide and nematode application.

Acknowledgements

I wish to thank my supervisor, Dr. Jean R. Finney-Crawley, for her guidance and interest throughout the duration of this project.

I also wish to acknowledge the kind encouragement given by Dr. Finney-Crawley's technical staff which included Sandra Osmond and Elizabeth Young.

I would like to thank Dr. Thakor Patel and his research assistant Donna Jackman for use of their laboratory during the microbiological portion of this research.

I wish to express considerable thanks to the research staff of Dr. Gordon Bennett, including Jennifer Caines and Madonna Bishop who provided invaluble advice during preparation of the manuscript, especially during data analysis. I would like to thank Nancy House and Sylvie Dupuis for help with the manuscript preparation.

I wish to express thanks to the School of Graduate Studies for providing financial assistance.

Finally, I would like to thank Janice Pitcher, who dissected hundreds of insect cadavers, typed a major portion of this thesis and who has given me encouragement over the duration of this project and throughout my academic career.

Table of Contents

List of Figures

List of Tables

Chapter 1

INTRODUCTION

The use of chemical insecticides has dramatically increased the yield of numerous commodities. Various problems such as insect resistance, resurgence of treated populations, elevation of secondary pests to primary status and the general pollution of the environment due to insecticide residues and subsequent non-target organism toxicity has made biological control a desired and vital alternative.

Biocontrol methods include release of exotic parasites and predacious insects, development of resistant plant varieties and dissemination of insect pathogens such as viruses, fungi, bacteria and nematodes. While control of pests by biological means has resulted in frequent successes, many pest problems cannot be solved by any single approach.

Integrated control is an approach to pest control which combines and integrates biological and chemical methods. Chemical control is used as necessary and in a manner which is least disruptive to biological methods. Integrated control may make use of naturally occurring biological control by manipulation or introduction of biotic agents (Shepard 1973).

An integrated control program should combine two or more techniques so they are more effective and less costly (including environmentally), than use of any one method. Successful pest management has many components and requires multidisciplinary approaches to pest problems. In addition to applied entomology, bioclimatology and parasitology, toxicology and studies on the effects of different kinds of pesticides on both harmful and beneficial species are essential. Better understanding of the mechanisms of toxicity and resistance to insecticides and ways of managing a population to avoid resistance must be undertaken.

Before an integrated control program can be undertaken the mortality factors already operating in the pest population should be evaluated and any supplemental method of control should be first examined to see if it is compatible with existing procedures. This is particularly pertinent for insecticide application. An insecticide may eliminate natural enemies in the ecosystem that exert control when the pest is below economic damage thresholds.

Members of the family Steinernematidae Chitwood and Chitwood are obligate parasites of insects. The host range of the steinernematid, *Steinernema feltiae* (syn. *Neoaplectana carpocapsae* Weiser) is extensive. Over 250 insect species from 10 orders are reported to serve as hosts (Poinar 1979). Comparatively few other entomogenous nematodes cause host mortality, with the majority of interactions being at most mildly debilitative (Gaugler 1981). Steinernematid nematodes do cause considerable host mortality and are thought to have tremendous biological control potential; however, many susceptibility tests have taken place in the laboratory and have not translated into field successes (Lewis

and Raun 1978; Gaugler 1981). Steinernematid nematodes have a number of attributes for biological control, including a wide host range, the ability to search for and kill an insect host within 48 hours, and ease of production. In addition, infective stages are easily stored and applied in the field and while there are a few reports of insect immunity, the organism is environmentally safe. Growth of steinernematids is inhibited above 30°C and as a result, parasitism of homoiotherms is unlikely (Kaya 1977).

There are a number of liabilities associated with the use of steinernematids, including natural enemies such as protozoa and soil-inhabiting fungi, but the major obstacle to the use of these agents for biocontrol is their moisture dependency. Thus, the majority of successful field trials have been confined to control of soil-dwelling insects. Soil applications have reduced field populations of root maggots (Cheng and Bucher 1972), root weevils (Harlan *et al.* 1971; Burman *et al.* 1979) and other soil-dwelling stages of insects (Poinar 1986).

These entomogenous nematodes have a non-feeding, free-living, infective juvenile stage, sometimes referred to as a "dauerlarva", and is naturally found in soil, where it is able to locate an insect host and enter the insect through the mouth, anus and spiracles, or by penetrating the cuticle (Triggiani and Poinar 1976; Schmidt and All 1979).

Infective stage juveniles are covered by a double cuticle. The cuticle is semi-permeable and is composed of a number of macromolecules such as albumins, glucoproteins and collagen that cover the entire external surface of the nematode

and serve as a barrier to the environmental milieu; it gives the nematode some resistance to its' environment (Poinar 1983). The cuticle contains a number of sense organs which are thought to be important in host-finding (Poinar 1986).

Infective stage juveniles carry a mutually associated bacterium in their intestine (Dutky 1959; Poinar 1966; Poinar and Thomas 1966,1967). The bacterium, *Xenorhabdus spp.*, is released by the nematode in the host haemocoel after the nematode sheds the outer cuticle (Schmiege 1963). Within the hemocoel, bacteria rapidly multiply, causing insect mortality through septicemia (Lysenko and Weiser 1974), within 24-48 hours (Dutky 1956). The bacteria provide nutrients for the nematode, while producing antibacterial compounds that suppress growth of other bacteria (Akhurst 1982,1986).

The nematodes develop into adult males or females and mate. Eggs develop and eventually break into the pseudocoelom of the female. When the nematodes have developed sufficiently, they break out of the mother and into the insect haemocoel (Schmiege 1963). This process repeats itself for a number of generations until nutrients are exhausted. Under suitable environmental conditions, infective juveniles leave the insect cadaver in search of another insect host. Up to 200,000 infective juveniles may emerge from the cadaver of an infected insect. However, the number of emerging nematodes varies according to insect size, nematode species and temperature of incubation (Gaugler 1981).

Infective larvae reared on natural hosts have been used to mass produce nematodes on artificial media. Several types of media have been developed, all of

which have a high protein content. Early success was accomplished by Glaser (1931) with *S. glaseri* on meat infusion plates and steinernematids have since been cultivated successfully on several different media (Dutky *et al.* 1964; House *et al.* 1965; Bedding 1976; Hansen and Hansen 1978; Hara *et al.* 1981; Wouts 1981; Bedding 1984). Infective juveniles can be stored for several months at low temperatures and remain infective in aerated liquid suspension or in shallow volumes of water (Lindegren *et al.* 1979).

In vitro rearing techniques can be used to study the effect of insecticides on the reproductive potential of local isolates of steinernematid nematodes. Because the culture method is monoxenic, in that it contains surface-sterilized nematodes and their associated bacterium, insecticide exposures must take cognizance of effects on the bacterial symbiont.

The bacterium, *Xenorhabdus nematophilus*, was originally isolated from infective juveniles by suspension of surface-sterilized nematodes in insect hemolymph (Poinar 1966) and cultured on nutrient agar plates. Thomas and Poinar (1979) proposed the genus *Xenorhabdus* to describe species of nematophilic bacteria. To date, one *Steinernema* sp. bacterial isolate has been identified, a non-luminescent gram negative, rod shaped bacterium, *Xenorhabdus nematophilus* (Akhurst and Boemare 1986). This species has only been isolated from steinernematids and their parasitized hosts.

Akhurst (1980) noted that there were two distinct forms of *X. nematophilus* from *S.feltiae.* He termed these the primary and secondary forms and discovered

that the primary form develops into deep blue colonies on nutrient bromothymol blue triphenyltetrazolium chloride agar, due to absorption of bromothymol blue, while the secondary form cannot absorb bromothymol blue and as a result, forms red colonies on the medium. During *in vitro* growth of steinernematid nematodes, the primary form of the bacteria is required for growth, whereas little growth occurs in the presence of the secondary form.

Although steinernematid nematodes have been cultured axenically, development and reproduction are significantly greater when the primary form of the associated bacterial symbiont is grown along with the nematodes (Bedding 1981). The technique of culturing nematodes along with bacteria is referred to as monoxenicity. Bacteria are able to convert the protein in the medium into suitable components for nematode development (Bedding 1976). While only two species of *Xenorhabdus* have been identified, *X. nematophilus* and *X. luminescens*, it is apparent that different strains occur and there exists a specific association between nematode isolate and bacterial strain (Akhurst 1983). In addition, steinernematid nematodes do not grow on strains of *X. nematophilus* obtained from other steinernematid strains or species (Akhurst 1983).

Steinernematid nematodes show considerable promise as biological control agents of soil insects because soil is their natural habitat (Gaugler 1981; Ishibashi and Kondo 1986). The ability of an entomogenous nematode to successfully infect soil-dwelling stages of insects depends on a number of factors, some of which include moisture, soil type, nematode migration and host susceptibility.

Soil type clearly has an effect on movement and subsequent infection of an insect host. Many factors influence movement of nematodes in soil and they appear to be interrelated; they include pore size, particle size, gravity, nematode activity, temperature, moisture gradient and size of nematode (Wallace 1958a,1958b,1960).

Burman and Pye (1980) have shown the movement of *S. feltiae* in response to temperature. Temperature probably plays a significant role in migration as Byers and Poinar (1982) showed that *S. feltiae* was capable of migrating toward slight (0.3°C) temperature gradients, comparable to those produced by insects in soil.

Host seeking ability by parasitic nematodes has received considerable attention in the past 10-15 years. Research on the mechanisms of chemoreception in entomogenous nematodes is generally lacking with most research focusing on plant-parasitic nematodes and the free-living nematode, *Caenorhabditis elegans* (Croll 1977). These receptors respond to chemical stimuli such as kairomones, which are chemical cues that induce a favourable response to the receiving organism such as in nematode-host attraction (Huettle 1986). Kairomones can take many forms such as sex pheromones, inorganic compounds, amino acids and carbon dioxide.

The actual chemoattractant to steinernematid nematodes has received attention in recent years and a number of agents have been implicated, such as larval excrement, fecal amino acids (Schmidt and All 1978,1979; Pye and Burman 1981) and carbon dioxide (Gaugler *et al.* 1980). It is likely that carbon dioxide is an

attractant as nematodes enter susceptible hosts through natural orifices including the spiracles, the sites of expiration of carbon dioxide. This is even more pertinent in chemoattraction in soil as gases such as carbon dioxide are able to move through air spaces 10^4 times faster than through water.

Pye and Burman (1981) have proposed interesting theories on the role of the chemical gradient in the life history of *S. feltiae*. They determined that the nematode was attracted to sodium and carbonate ions as well as gram negative bacteria. Plants, which can accumulate these ions, release these to the environment when being fed upon by insects. Bacterial growth occurs at this site, with metabolism of plant carbohydrates releasing carbonate. These biochemicals serve to attract the entomogenous nematode to the host insect.

The tendency for many soil-inhabiting entomogenous nematodes is to migrate towards the soil surface (Schroeder and Beavers 1987). Alternatively, a number of studies have shown the ability of entomogenous nematodes to move downwards or towards, an insect host(Georgis and Poinar 1983a,1983b, 1983c). At present little information is available regarding the effect of insecticides on the migration of entomogenous nematodes in soil and therefore this aspect was investigated in this study.

Many physicochemical processes determine the fate of an insecticide in soil; the major factor is adsorption (Kahn 1980). Adsorption of an insecticide on soil particles depends upon the soil type and the chemical characteristics of the insecticide molecule. Solubility of an insecticide in water is an approximate

indicator of adsorption to soil particles. An inverse relationship between solubility and adsorption has been observed, in that a chemical with high water solubility has low hydrophobic character and consequently, adsorption to soil is low (Kahn 1980).

Soil-migrating nematodes move through the water film in the pores of soil (Wallace 1960). Therefore, maximum insecticide-nematode contact occurs in that water film. Strongly adsorbing insecticides are absent from the water film and thus, bioavailability is low (Steele and Hodges 1975).

The potential for use of steinernematid nematodes against soil-dwelling insect pests is evident and as chemical insecticide use is still generally popular, the integration of the two methods may be economically productive as well as environmentally sound.

While the compatibility of various herbicides and insecticides with plant-parasitic nematodes has received considerable attention (Greco and Thomason 1980; Marban-Mendoza and Viglierchio 1980a,1980b,1980c; Peacock and Dunn 1986), the interaction of such compounds with entomogenous nematodes is incompletely understood. Several reports of general compatibility at levels of commercial pesticide use have been carried out (Fedorko *et al.* 1977a; Kamionek 1979), yet these tests have only directly exposed the nematodes, usually by immersion in an insecticide solution. Other research has focused on the development of nematodes in an insect host after insecticide exposure (Hara and Kaya 1983a,1983b) or incorporation of insecticides into agar medium for *in vitro*

reproduction of the nematode (Hara and Kaya 1982). Little attention has been given to the effect of insecticides on host seeking ability of steinernematid nematodes in soil and to date, no work has been undertaken to determine the effects on Newfoundland isolates.

In addition to examining the effect of selected insecticides on survival and *in vivo* and *in vitro* development, the present study seeks to determine the effect on host seeking ability of two local entomogenous nematode isolates from Newfoundland in insecticide-treated soil.

The objectives of this study are to determine whether combinations of chemical insecticides with nematodes will enhance the action of both agents, or whether the chemical insecticide will adversely affect the nematode. Specifically, the following points were examined:

- To examine the direct toxicity of selected insecticides on nematode isolates
- To ascertain the result of direct insecticide exposure on the efficacy of infective juvenile nematodes toward a suitable insect host.
- To determine the effect of selected insecticides on *in vitro* nematode reproduction.
- To determine the effect of selected insecticides on the mutualistically associated bacterium of both nematode isolates.
- To determine migration of nematodes in insecticide-treated soil.

Chapter 2

MATERIALS AND METHODS

2.1. ORGANISMS

2.1.1. *Galleria mellonella*

Galleria mellonella was reared according to the method of Dutky *et al.* (1962). Late instar larvae were used for the *in vivo* production of nematodes and for all subsequent experimental procedures requiring an insect host.

2.1.2. Nematodes

Two entomogenous nematodes, isolated by Finney (1984) and tentatively identified as *Steinernema* sp. Newfoundland strain No. 1 or L1C and *Steinernema* sp. Newfoundland strain No. 2 or 5B, were used in these experiments. Each nematode was propagated by passage through *G. mellonella* at 17°C (Dutky *et al.* 1964). Infective juvenile nematodes were stored at 5°C in shallow, distilled water in tissue culture flasks. All juvenile nematodes were less than two months old and had double cuticles when utilized in experiments.

2.1.3. Isolation of Bacteria

Infective juveniles of both L1C and 5B were surface-sterilized by placing them in a sterile solution of 0.05% Hyamine for 30 minutes (Poinar 1966). The bacterium from each nematode was releasede by macerating a concentrated nematode suspension with a sterile mortar and pestle. Samples from the macerate were streaked on nutrient bromothymol blue triphenyltetrazolium chloride agar (NBTA). Isolated colonies were picked from plates and restreaked on NBTA until pure colonies of the bacteria were obtained. Colonies appeared on NBTA plates within 3-5 days when incubated at 15°C. Symbiotically-associated bacteria of both L1C and 5B were tentatively identified as *Xenorhabdus* sp. isolate L1C and *Xenorhabdus* sp. isolate 5B and were known to be dimorphic (Akhurst 1980). Bacteria were maintained on NBTA plates at 15°C and routinely transferred every 7-10 days.

2.2. INSECTICIDES

Seven insecticides were chosen to encompass the major categories of pesticides. These included 2 organochlorine insecticides (Chlordane, Methoxychlor), 2 organophosphates (Malathion, Diazinon), 2 carbamate insecticides (Carbaryl(Sevin^R), Aminocarb (Matacil^R)) and an insect growth regulator (Diflubenzuron(Dimilin^R)). The organochlorine and organophosphate insecticides as well as carbaryl were commercial formulations from Chipman Inc. Limited. Carbaryl was a water soluble miscible liquid while the other formulations were emulsifiable concentrates. Dimilin and Matacil (1.8D OSC(T2)) were technical grade material. Dimilin was a powder with 99 % active ingredient. Matacil was formulated in oil (Ultramar stove oil) with 19.9 $\pm$ 0.5% active ingredient.

The commercial formulations were used as stock solutions with the manufacturers guarantee used for insecticide concentration content. The technical grade Matacil was used as a stock solution. A stock solution of diflubenzuron was obtained by dilution with reagent grade dimethylsulfoxide (DMSO) from BDH chemicals. Insecticide stock solutions had the following concentrations; chlordane, 400 mg/ml; methoxychlor, 288 mg/ml; malathion, 500 mg/ml; diazinon, 62.5 mg/ml; carbaryl, 150 mg/ml; Matacil, 175.8 mg/ml; Dimilin, 100 mg/ml.

2.3. ACUTE TOXICITY OF INSECTICIDES TO NEMATODES

2.3.1. Behaviour Effects due to Direct Exposure to Insecticides

Glass petri dishes (60 x 15 mm) were filled with 9.0 ml of appropriately concentrated insecticide. A stock solution of infective juvenile nematodes was made to a final concentration of 1000 per ml of water. The insecticide solutions were formulated such that when 1.0 ml of stock nematode solution was added, final insecticide concentrations were 1.0, 0.1, 0.01 and 0.001 mg/ml. Untreated and solvent-treated nematode suspensions (1% v/v) served as controls.

Behaviour of juvenile nematodes, as described by Hara and Kaya (1983a), was assessed after exposure to insecticides for 24, 72, 120 and 168h. To assess nematode response, each petri dish was swirled several times to make an even suspension of nematodes. A pipette was used to remove 0.5 ml of nematode suspension to a clean petri dish and the response of 25 nematodes was assessed by mechanical stimulation with a probe.

Tests were conducted at 15°C and each insecticide concentration was replicated three times with 25 nematodes per replicate. Untreated controls were also examined.

2.3.2. Infectivity Effects due to Direct Exposure to Insecticides

Insecticide stock solutions were diluted in 1.5 ml distilled water such that the addition of 1.0 ml of stock infective juvenile solution (1000/ml) yielded final insecticide concentrations of 1.0, 0.1, 0.01 and 0.001 mg/ml. Both insecticide and nematode suspensions were added together in a glass test tube (16 x 125 mm) and incubated at 15°C for the incubation period. At 24, 72, 120 and 168h, 0.62 ml of suspension, containing ca. 250 nematodes, were pipetted onto filter paper at the bottom of a petri dish (60 x 15 cm). Five *G. mellonella* larvae were placed on the filter paper and exposed to insecticide-treated nematodes for 48h at 15°C. After incubation, the larvae were washed with distilled water and placed on fresh, moistened filter paper. Five to six days after initial exposure, alive and dead larvae were enumerated. Dead larvae were autopsied and observed for presence of nematodes. Infectivity tests were conducted with all insecticides based on behaviour data. Larvae exposed to diazinon, chlordane and methoxychlor were dissected and adult nematode(s) counted. The mean number of adult nematodes per infected *G. mellonella* larva was referred to as the Infection Index (Alikhan *et al.* 1985). Each insecticide treatment was replicated three times at each concentration and time exposure, with five insects per replicate. Baseline Infection Index was determined with untreated nematodes. Infection Index data were arcsine transformed and subjected to analysis of variance and Student-Newman-Keuls(SNK) test procedure. Nematode scores after dissection were subjected to analysis of variance and SNK test procedure (Sokal and Rohlf 1969).

2.4. TOXICITY OF INSECTICIDES TO SYMBIOTIC BACTERIA

2.4.1. Effect of Insecticide on Generation Time

2.4.1.1. Bacterial Medium

Growth medium contained 16.0g nutrient broth, 2.0g yeast extract and 1.0L distilled water.

A 125 ml volume was dispensed into 500 ml flasks and autoclaved at 121°C for 15 minutes. Media were cooled to room temperature. These flasks were referred to as "experimental flasks".

2.4.1.2. Generation Time Determination

A 2L flask containing 250 ml of medium was inoculated with culture from NBTA plates and incubated overnight at 15°C using a shaking psychrotherm (New Brunswick Scientific, Co.) to obtain log phase culture. A sterile pipette was used to inoculate a volume of the log phase culture into fresh experimental flasks until just turbid. Flasks were incubated at 15°C using a shaking psychrotherm (90-100 rpm). At time zero and subsequently every two hours, a 1.0 ml aliquot of culture was aseptically withdrawn and the optical density (O.D.) was spectrophotometrically measured at 600 nm (Bausch and Lomb, Schimadzu UV-260 Spectrophotometer). Uninoculated flasks containing insecticide were also incubated and optical density measured to serve as controls.

A calibration curve was used to correct observed optical density values to true optical density (Lawrence and Maier 1977). An aliquot of 100 ml of the overnight

log phase culture was centrifuged at 12,000g for 15 minutes using a Sorval RC-5 centrifuge. The pellet was resuspended in 0.15 M NaCl. Centrifugation and washing was repeated three times. After final centrifugation, the pellet was resuspended in 2.0 ml of 0.015 M NaCl and 0.2 ml of of this thick suspension dispensed into a 9.8 ml blank. Five milliliters of vortexed suspension was transferred to a new 5.0 ml saline blank. This was repeated six additional times. The optical density at 600 nm was measured for each dilution. These values were used to calculate a unit O.D. and this value was used to correct the observed optical density readings (Lawrence and Maier 1977).

Experiments were replicated four times. Mean optical density was plotted against each time interval. The generation time was determined using the following formula (Schlegel 1986);

$$G.T. = \frac{t \log 2}{\log O.D._A - \log O.D._B}$$

where t is the time (in minutes) elapsed and $O.D._A$ and $O.D._B$ represent optical density readings at two times during incubation. The time period of optimal growth was determined from mean optical densities. Generation time was determined by applying the formula to each of the four replicates over the optimal period. The mean of these four values represented the generation time of the bacterium in question. Generation times were logarithmically transformed followed by analysis of variance and SNK test procedure for significant differences among means.

2.4.1.3. Insecticide Incorporation

All insecticides excessively clouded the medium at 1.0 and 0.1 mg/ml such that optical density measurement was prohibited. Malathion did not cloud medium at 0.1 mg/ml. Generation time was determined at insecticide concentrations of 0.01 and 0.001 mg/ml for all insecticides along with the malathion exception.

Stock insecticide solutions were exposed to ultraviolet light for 15 minutes prior to addition to experimental flasks. Flasks were shaken overnight at 15°C to dissolve insecticide before inoculation with bacteria. Experiments were replicated four times and data evaluated as noted above.

2.4.2. Effect of Insecticide on Growth on Solid Medium

2.4.2.1. Medium/Insecticide Incorporation

Media were treated identically to those used in generation time experiments, with the addition of 1.5% agar. Sterile stock solutions of insecticide were added to the medium after autoclaving and allowed to cool to room temperature. Viability of bacteria were tested at 1.0, 0.1, 0.01 and 0.001 mg/ml of each chemical.

2.4.2.2. Bactericidal Activity of Insecticides

A thick cell suspension, similar to that used during *in vitro* reproduction tests, was made in nutrient broth. A dilution series was made by dispensing 1.0 ml of undiluted culture into a 9.0 ml sterile Ringer solution blank. This was carried out five more times so that a series of dilutions ranging from undiluted to 10^{-6} were made. The spread plate technique was used to inoculate 0.1 ml of suspension from each dilution on culture plates (Pelczar and Reid 1972). After six days incubation at 15°C, colonies were enumerated and tabulated at all dilutions.

Culture plates with less than 300 colonies were used to assess survival of bacteria. Accurate counts could not be made when plates contained in excess of 300 colonies. All dilutions were plated. Only those producing countable colonies were utilized in experiments. The number of colonies on control and insecticide treated plates were compared. The experiment was carried out twice with two replicate plates per repeat for all insecticides at 1.0, 0.1, 0.01 and 0.001 mg/ml.

In a similar experiment, an undiluted bacterial cell suspension was applied to insecticide-treated nutrient plates; the insecticide concentration failing to produce visible colonies was noted. The experiment was carried out three times with two replicate plates per repeat for all insecticides at 1.0, 0.1, 0.01 and 0.001 mg/ml for both bacteria. Untreated and solvent-treated DMSO controls (1% v/v) were also assessed.

2.5. EFFECT OF INSECTICIDES ON IN VITRO REPRODUCTION OF NEMATODES

2.5.1. Nematode Beef-Liver Medium

A synthetic medium was developed to promote *in vitro* growth of the entomogenous nematodes. This medium was termed Beef-Liver medium (BL medium) and contained 8.0g nutrient broth, 5.0g beef extract, 5.0g liver extract, 15.0g Bacto-Agar and 1.0L distilled water. The medium was boiled to dissolve the nutrients and 250 ml of medium was dispensed in 500 ml flasks. Media were autoclaved under pressure at 121°C for 15 minutes and then cooled to 50°C before addition of insecticide.

2.5.2. Insecticide Incorporation

Open glass vials of insecticide stock solution and/or appropriate dilutions of insecticide were placed under ultraviolet light in a containment cabinet for 15 minutes. An appropriate volume of insecticide was added to each flask to give final insecticide concentrations of 1.0, 0.1, 0.01 and 0.001 mg/ml of BL medium. A 30-35 ml volume of medium was poured from each flask into six plastic petri plates (100 x 15 mm), and cooled to room temperature.

2.5.3. BL Agar Plate Inoculation

A suspension of nematodes was centrifuged at 300 rpm for five minutes to pellet the nematodes into a thick suspension. Nematodes were resuspended in a sterile centrifuge tube with sterile 0.05% Hyamine and incubated for 30 minutes at 15°C with frequent agitation to accomplish the surface sterilization. Following incubation, nematodes were washed three times in sterile Ringer solution (Barka and Anderson 1965). The concentration of nematodes was adjusted to give 100 juveniles per 0.1-0.3 ml of Ringer solution.

A thick suspension of the associated bacteria was made in sterile nutrient broth and swabbed over the BL medium.

One hundred surface sterile nematodes were pipetted upon the surface of the BL agar and the plate sealed with a Parafilm^{R} strip. Plates were incubated at 15°C for 14 days.

2.5.4. Nematode Recovery from BL Medium

At 14 days post-inoculation, nematodes were recovered from each plate by flooding 10 ml of Ringer solution on each plate and swirling for 10-20 seconds. The suspension was poured into a plastic petri dish and adults and juveniles were either counted in total or, in the case of a dense population, diluted in a graduated cylinder and the population estimated from values obtained from the average of three aliquots. Toxicity to nematode reproductive potential was assessed at 1.0, 0.1, 0.01 and 0.001 mg/ml with three repeats at each concentration and six replicates per repeat. Control growth, in the absence of insecticide, was also determined. A DMSO solvent control was assessed at 1% (v/v). Nematode population was scored from 1 to 7 based on the number of juveniles. A score of 1 indicated the juvenile population did not exceed the initial inoculum. A score of 2 indicated more than 100 but fewer than 500 juveniles, up to a score of 7 which indicated maximum nematode development and reproduction (Table 3-5). This scoring method was adapted from Hara and Kaya (1983a). Population scores were subjected to Kruskal-Wallis analysis of variance and non-parametric multiple comparisons (Zar 1984).

2.6. MIGRATION OF NEMATODES IN SOIL

2.6.1. Soil Column Preparation

Five 2 cm (deep) x 5 cm (diameter) plastic rings were fastened together with adhesive tape to form a 10 cm vertical cylinder. Ten late instar *G. mellonella* larvae were secured at the base of the cylinder with several layers of cheesecloth and elastic bands. Pro-Mix^{R}, presterilized soil was sifted through a Canadian

Standard sieve to give soil with particle size below 850μm. Soil was dried overnight at 50°C, then mixed with distilled water in a 3:1 ratio until homogeneous. Cylinders were filled with 90-100g of soil mixture, the soil was allowed to settle by gentle tapping of the cylinder against the work bench. Cylinders were covered with aluminum foil, to prevent evaporation, and held vertically at 15°C overnight. Columns without *G.mellonella* at the base served as controls.

2.6.2. Column Inoculation

Stock solutions of either L1C or 5B nematodes were adjusted to yield a final concentration of nematodes of 3000 per 0.5-1.0 ml.

Aluminum foil was removed from the top of the cylinder, a small amount of soil cleared to the perimeter, and 3000 nematodes inoculated into the soil. The soil was pushed gently back into place and the foil cover replaced. Cylinders were incubated vertically at 15°C for 48h.

2.6.3. Enumeration of Migrated Nematodes

Adhesive tape was carefully removed from the top cylinder ring and a sheet of paper inserted between layers. The initial layer of soil (0-2 cm) was removed and placed on cheesecloth which was then fastened with an elastic band. The soil was placed in a Baermann funnel and left overnight at 17°C (Goodell 1982; Hooper 1986). A sufficient volume of distilled water was added to cover the soil. The remaining four layers of 2 centimeters each were recovered in a similar manner.

Approximately 20-25 ml of water was collected from each funnel and all nematodes counted.

Additionally, *G. mellonella* recovered from each cylinder were placed in a petri dish on moistened filter paper. After five to seven days, these larvae were dissected and adult nematodes counted. Experiments were replicated ten times per nematode species.

2.6.4. Insecticide Incorporation

Insecticide was mixed with distilled water before addition to dry soil. Chlordane and diazinon were tested at 1.0, 0.1, 0.01 and 0.001 mg/ml distilled water with both nematodes (see 2.6.2). The experiments were replicated five times per nematode species. Columns without insects served as controls.

Data were square-root transformed followed by analysis of variance and SNK test procedure (Sokal and Rohlf 1969).

Chapter 3

RESULTS

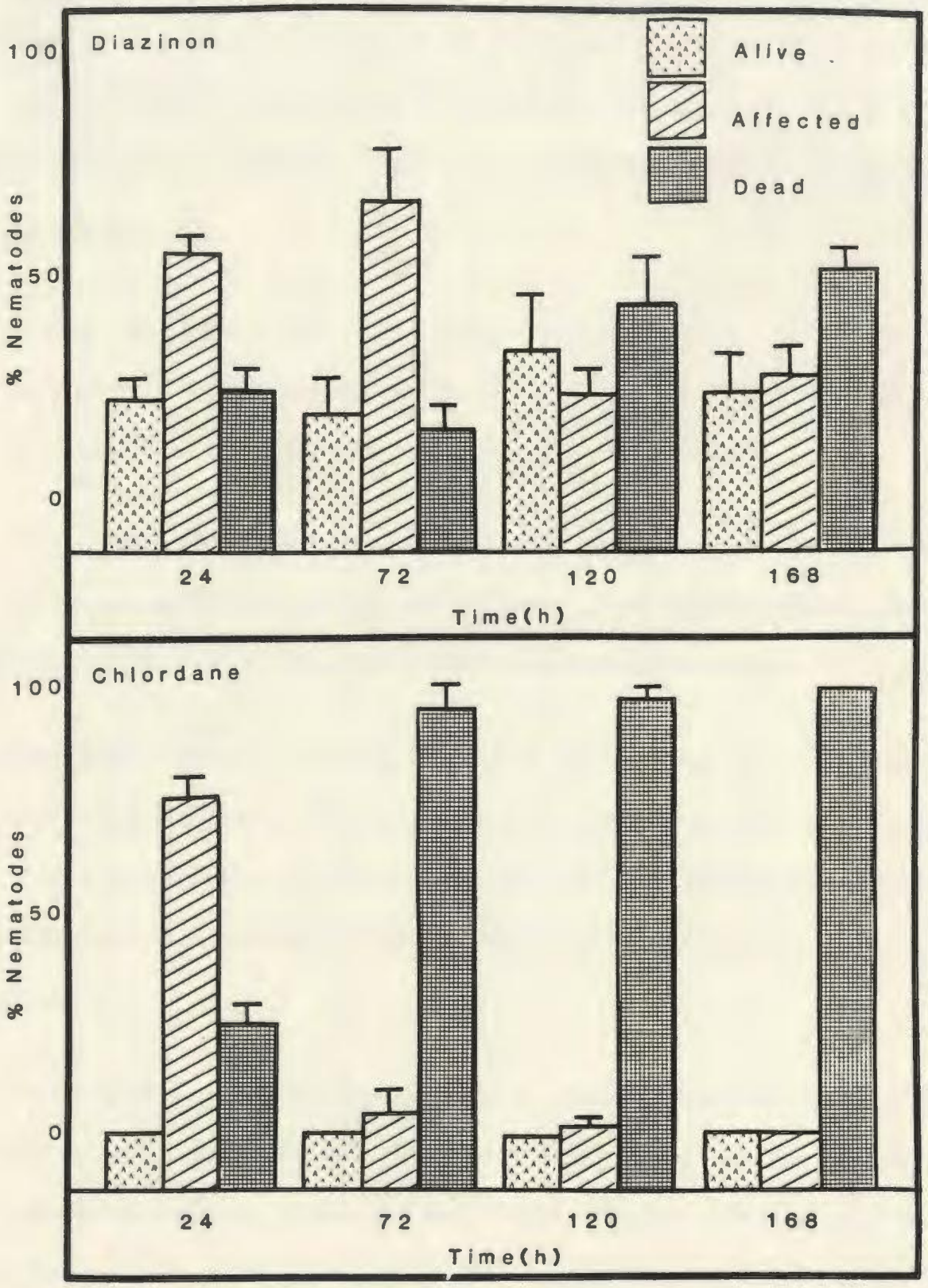
3.1. ACUTE TOXICITY OF INSECTICIDES TO NEMATODES

3.1.1. Toxicity due to Direct Exposure of Insecticide

Condition of nematodes after exposure to insecticides was categorically divided into three groups; alive, affected and dead. Affected or inactive nematodes typically had a J-shaped posture and responded weakly to mechanical stimulation with a blunt, metal probe. However, for both L1C and 5B juvenile nematodes, this state occurred infrequently over all time periods and insecticide concentrations. Dead nematodes had a straight posture and did not respond to mechanical stimulation.

A proportionately large number of affected nematodes were only found for 5B nematodes for chlordane and diazinon at 1.0 mg/ml. Figure 3-1 shows that at 1.0 mg/ml diazinon, there was a change in the percentage of affected nematodes over the time course from 54.7% to 26.7% while the percentage of dead nematodes increased from 24.0% to 50.7%. The proportion alive showed little change over the 168h incubation time (21.3-22.7%). At 1.0 mg/ml chlordane, the effect was more pronounced. Affected nematodes composed 76.0% of the 24h sample and dead nematodes 24.0%; by 72h 96.0% were dead and affected made up only 4.0%. At the conclusion of the incubation time, there was 100.0% mortality.

Figure 3-1: Percentage of *Steinernema* sp. Newfoundland strain no. 2 (5B) juveniles alive, affected or dead after exposure to 1.0 mg/ml chlordane and 1.0 mg/ml diazinon. Sample statistics are mean values $\pm$ SD. Three replicates with 25 nematodes per replicate.


Diazinon
Alive
Affected
Dead
100
50
0
% Nematodes
24
72
120
168
Time(h)
Chlordane
100
50
0
% Nematodes
24
72
120
168
Time(h)

The toxic effect of the organochlorine insecticides, chlordane and methoxychlor on 5B nematodes is indicated in Fig. 3-2. Concentrations of both insecticides at or below 0.1 mg/ml had a negligible effect. Clearly, at 1.0 mg/ml, both chemicals exert a toxic effect on the nematode. Mortality of 5B rose from 24.0% to 96.0% after exposure to chlordane, while methoxychlor was lethal to 80.0% of the population by 72h.

Figure 3-3 depicts the toxic effect of concentration on 5B by the organophosphate insecticide, diazinon. The chemical was toxic at 1.0 mg/ml as there was 50.7% mortality while only 22.7% were alive (Fig. 3-1).

The other organophosphate insecticide, malathion, and the carbamate insecticides, carbaryl and Matacil and the insect growth regulator Dimilin, showed no observable effect on 5B nematodes over all tested concentrations.

The organochlorine insecticides, chlordane and methoxychlor were toxic to juvenile L1C nematodes. Figure 3-4 shows that mortality at 24h was above 50% for both chemicals at 1.0 mg/ml and rose above 75.0% at 168h for both chlordane (85.3%) and methoxychlor (77.3%). At and below 0.1 mg/ml there was no toxic effect.

The organophosphate insecticide, diazinon, showed toxicity to L1C over the time course of the experiment (Fig. 3-5). There was no mortality at 24h but mortality increased to 45.3% by 168h. Unlike 5B, the carbamate insecticide, carbaryl, exerts a toxic effect on L1C. There was 13.3% mortality at 24h and this rose to 22.7% by 168h for 1.0 mg/ml. As well, there was some mortality (8.0%)

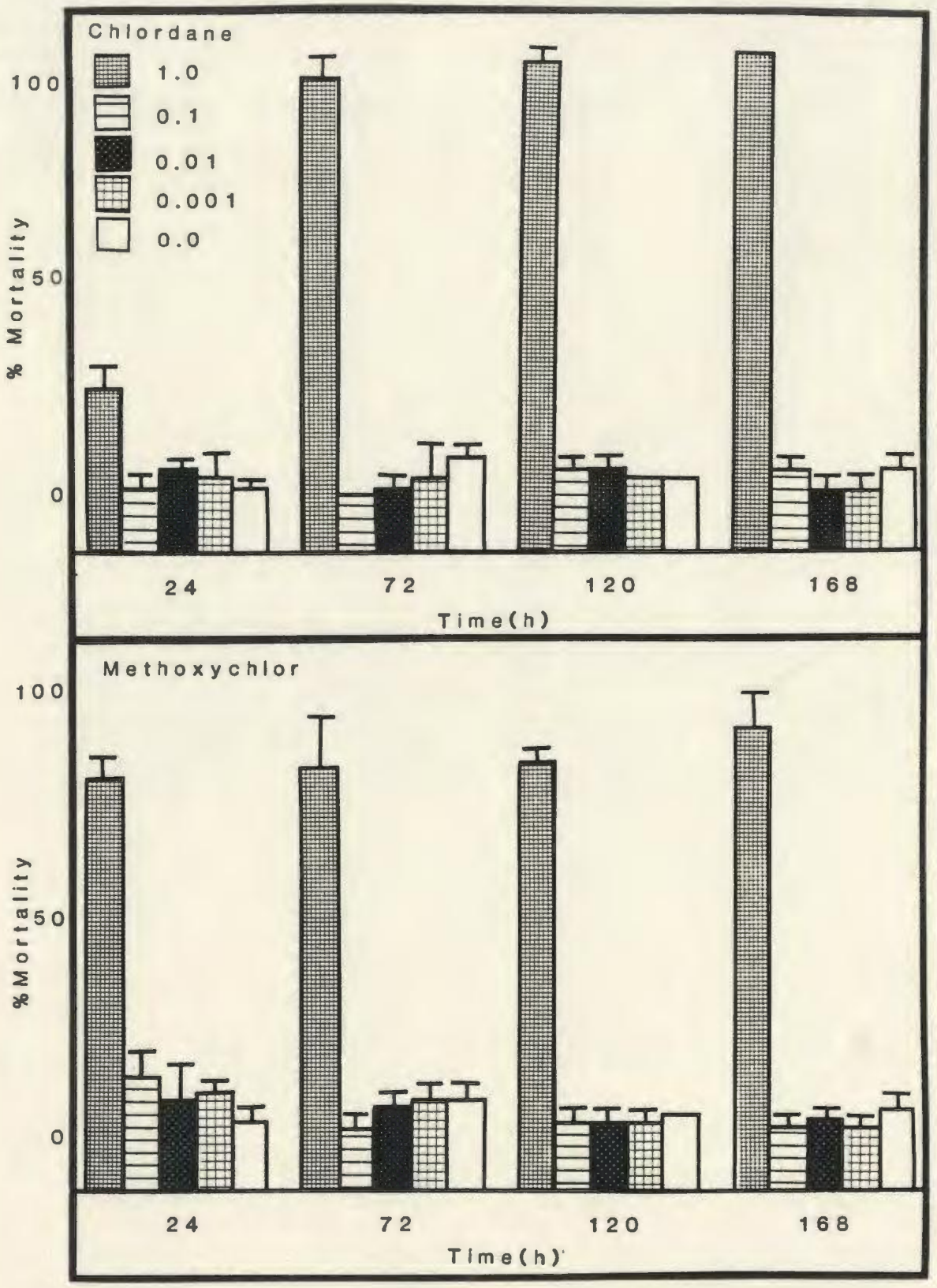
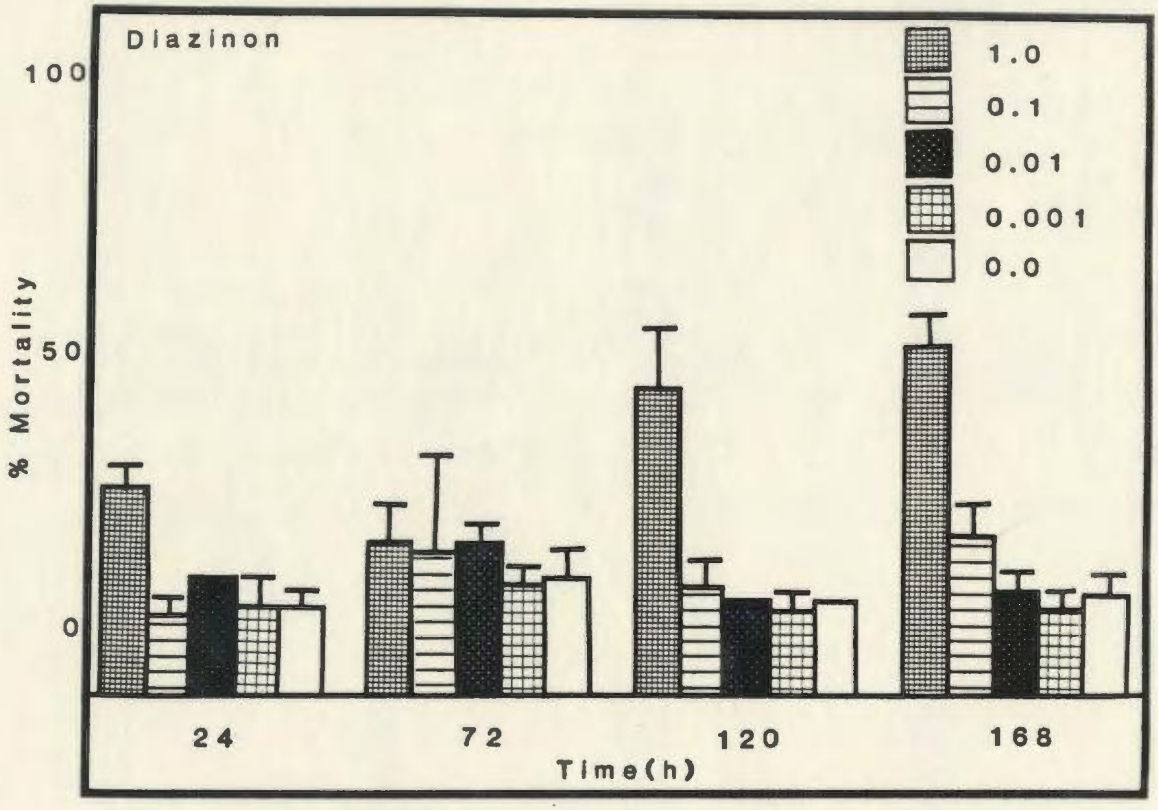
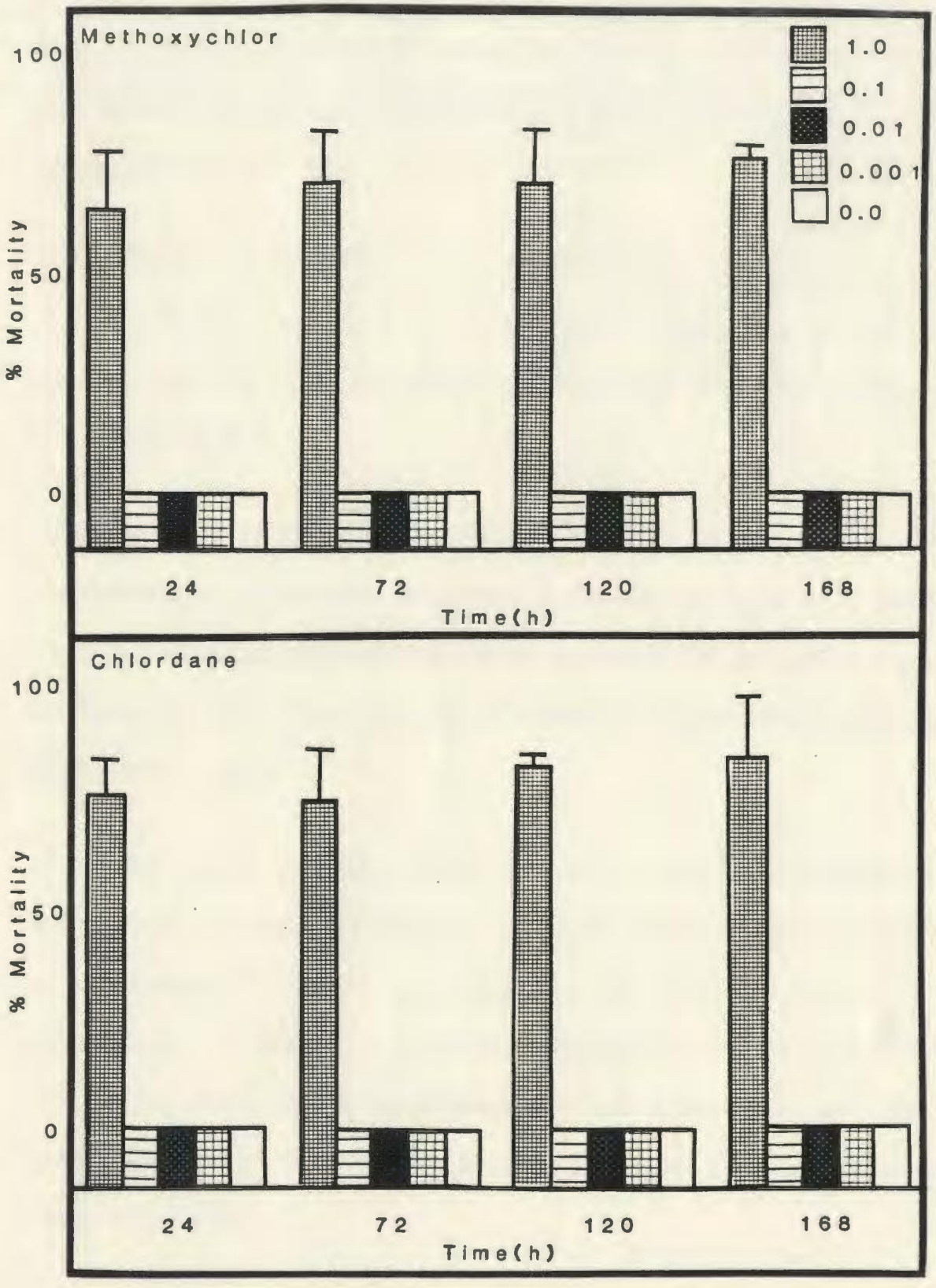

Chlordane
1.0
0.1
0.01
0.001
0.0
100
50
0
% Mortality
24
72
120
168
Time(h)
Methoxychlor
100
50
0
%Mortality
24
72
120
168
Time(h)

Figure 3-3: Percent mortality of *Steinernema* sp. Newfoundland strain no. 2 (5B) juvenile nematodes after exposure to the organophosphate insecticide, diazinon. Insecticide concentrations were as indicated, mg/ml. Sample statistics are mean values $\pm$ SD. Three replicates with 25 nematodes per replicate.


Diazinon
100
50
0
% Mortality
1.0
0.1
0.01
0.001
0.0
24
72
120
168
Time(h)


Methoxychlor
1.0
0.1
0.01
0.001
0.0
100
50
0
% Mortality
24
72
120
168
Time(h)
Chlordane
100
50
0
% Mortality
24
72
120
168
Time(h)

for 1.0 mg/ml at 168h. Although mortality was low, it was significantly higher than the lower carbaryl concentrations and controls. Dimilin, Matacil and malathion were not toxic to L1C juvenile nematodes at $\leq$1.0 mg/ml. Thus, the toxic effects of certain insecticides were usually at concentrations of 1.0 mg/ml for both nematodes.

3.1.2. Efficacy of Nematodes After Insecticide Exposure

Infection of *G. mellonella* larvae by 5B juvenile nematodes was significantly lower ($P<0.05$) at 1.0 mg/ml chlordane after 72h and at 1.0 mg/ml methoxychlor after 24h (Table 3-1).

High rates of nematode mortality lead to poor infection success. This relationship was evident upon comparison of Fig. 3-3 and Table 3-1. Table 3-1 shows that the organophosphate insecticide malathion, the carbamates, carbaryl and Matacil as well as the insect growth regulator Dimilin, did not significantly affect infection success.

Table 3-2 shows Infection Indices for 5B at varying concentrations of methoxychlor, chlordane and diazinon. Generally, Infection Indices for 5B were not significantly different at $\leq$0.1 mg/ml for all three insecticides over the incubation period. There was a significant reduction (cf. controls) in the Infection Index of nematodes exposed to diazinon and methoxychlor for 120h. Such a significant effect was not shown by nematodes that had been exposed to these insecticides for 168h.

Comparison of Fig. 3-2 and Table 3-2 for chlordane at 1.0 mg/ml shows that at

Figure 3-5: Percent mortality of *Steinernema* sp. Newfoundland strain no. 1 (L1C) after exposure to the organophosphate diazinon and carbamate insecticide, carbaryl. Insecticide concentrations were as indicated, mg/ml. Sample statistics are mean values $\pm$ SD. Three replicates with 25 nematodes per replicate.

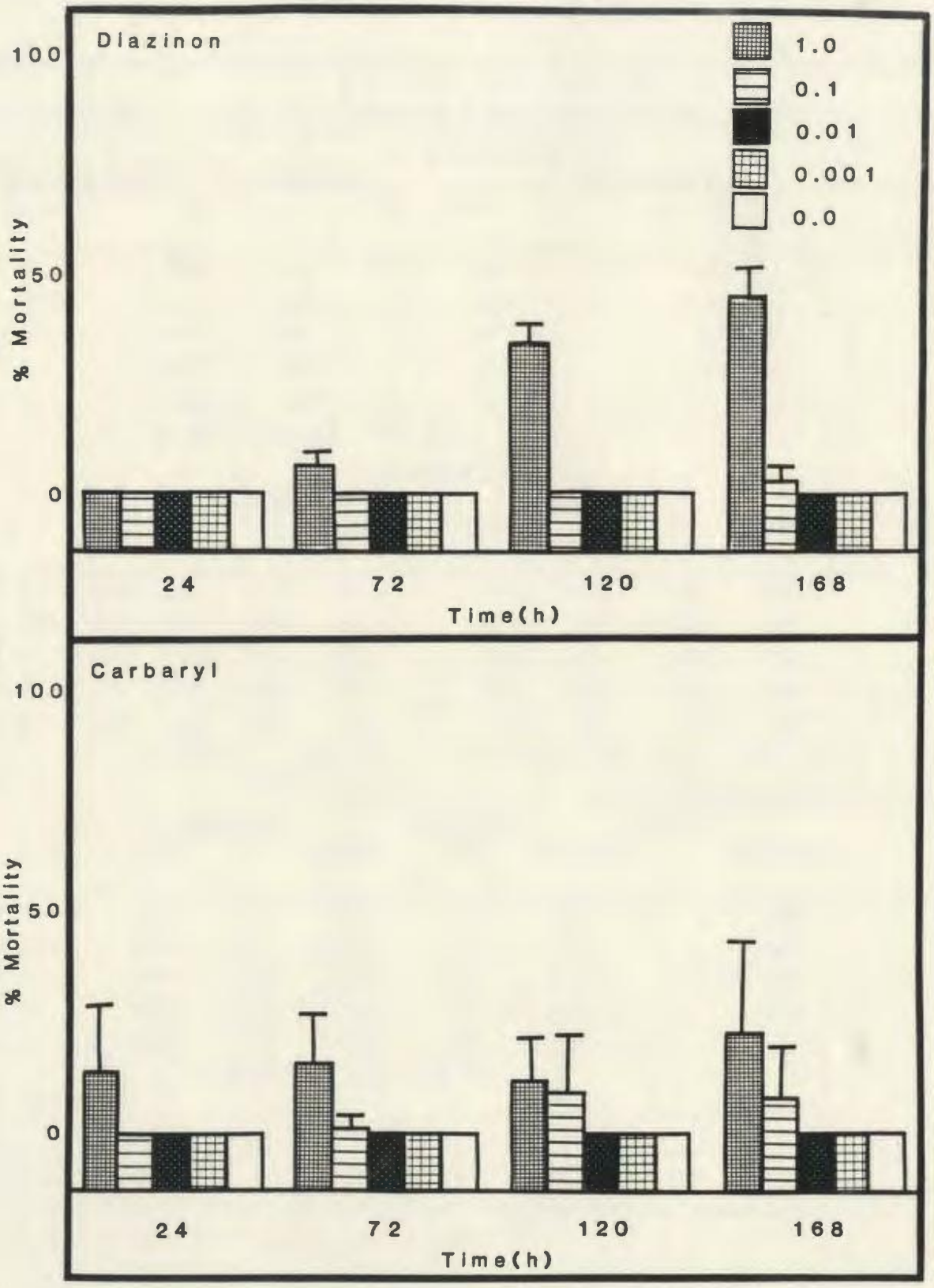

Diazinon
1.0
0.1
0.01
0.001
0.0
100
50
0
% Mortality
24
72
120
168
Time(h)
Carbaryl
100
50
0
% Mortality
24
72
120
168
Time(h)

Table 3-1: Recovery of *Steinernema* sp. Newfoundland strain no. 2 (5B) after exposure to insecticides and subsequent infection in *G. mellonella* larvae.[+]

Concentration (mg/ml)	% *G. mellonella* with nematodes after exposure(h) to insecticides							
	Organochlorines							
	Chlordane				Methoxychlor			
	24	72	120	168	24-168			
0.0	100	100	100	100	100			
0.001	100	100	100	100	100			
0.01	100	100	100	100	100			
0.1	100	100	100	100	100			
1.0	80 (0.0)	13* (11.5)	13* (11.5)	0*	0*			
	Organophosphates							
	Malathion				Diazinon			
	24	72	120	168	24	72	120	168
0.0	100	100	100	100	100	100	100	100
0.001	100	100	100	100	100	100	100	100
0.01	100	100	100	100	100	100	100	100
0.1	100	100	100	100	100	100	100	100
1.0	100	93 (11.5)	100	93 (11.5)	93 (11.5)	93 (11.5)	40* (20.0)	60* (20.0)
	Carbamates				Insect Growth Regulator			
	Carbaryl			Matacil	Dimilin			
	24	72	120	168	24-168			24-168
0.0	100	100	100	100	100			100
0.001	100	100	100	100	100			100
0.01	100	100	100	100	100			100
0.1	100	100	100	100	100			100
1.0	100	93 (11.5)	80 (0.0)	87 (23.1)	100			100
0.0(S.C.)[++]		-	-	-	-			100

[+]Combined data of three replicates with five larvae per replicate. Standard deviations are bracketed. Standard deviation was ±0.0 unless indicated.

[++]Solvent-treated control (1% v/v).

*Significantly different ($P<0.05$) from control by SNK test.

Table 3-2: Recovery of *Steinernema* sp. Newfoundland strain no. 2 (5B) from *G. mellonella* by dissection after exposure to selected insecticides. Sample statistics are mean ± SD.[+]

Insecticide Conc. (mg/ml)	Mean No. Nematodes per Larvae[++] 24h	72h	120h	168h
Methoxychlor				
1.0	0.0 (0) aA	0.0 (0) aA	0.0 (0) aA	0.0 (0) aA
0.1	6.9 ± 3.2(15) aB	3.7 ± 1.5(15) bB	4.1 ± 1.4(15) bB	4.7 ± 1.3(15) bB
0.01	6.3 ± 2.5(15) aB	4.2 ± 2.7(15) aB	6.1 ± 2.0(15) aC	4.5 ± 1.3(15) aB
0.001	5.3 ± 1.9(15) aB	3.3 ± 1.6(15) bB	5.3 ± 1.4(15) aBC	4.5 ± 1.4(15) aB
0.0	4.8 ± 2.4(15) aB	4.7 ± 1.9(15) aB	5.9 ± 2.3(15) aC	5.7 ± 2.3(15) aB
Diazinon				
1.0	6.5 ± 2.0(11) aA	3.9 ± 1.9(14) bA	1.5 ± 0.8 (6) cA	1.2 ± 0.4 (9) cA
0.1	6.7 ± 1.7(15) aA	3.4 ± 1.3(15) bA	4.2 ± 1.6(15) bB	3.3 ± 1.2(15) bB
0.01	5.5 ± 2.2(15) aA	3.3 ± 1.1(15) bA	5.1 ± 2.1(15) aBC	4.2 ± 1.7(15) abC
0.001	5.4 ± 2.3(15) aA	3.3 ± 1.4(15) bA	3.9 ± 1.2(15) bB	4.2 ± 1.7(15) abB
0.0	4.8 ± 2.4(15) aB	4.7 ± 1.9(15) aB	5.9 ± 2.3(15) aC	5.7 ± 2.3(15) aB
Chlordane				
1.0	5.0 ± 1.7(11) aA	0.0 (0) bA	1.0 ± 0.0 (2) bA	0.0 (0) bA
0.1	4.5 ± 2.2(15) aA	4.5 ± 1.7(15) aB	4.9 ± 1.9(15) aB	5.8 ± 2.9(15) aB
0.01	4.0 ± 2.1(15) aA	5.3 ± 1.9(15) aB	5.3 ± 2.1(15) aB	5.6 ± 2.5(15) aB
0.001	3.9 ± 1.9(15) aA	4.8 ± 2.1(15) abB	6.1 ± 2.2(15) bB	5.4 ± 2.5(15) abB
0.0	4.8 ± 2.4(15) aB	4.7 ± 1.9(15) aB	5.9 ± 2.3(15) aC	5.7 ± 2.3(15) aB

[+]Sample size is bracketed

[++]Means followed by different lowercase letters in a row and by different uppercase letters in a column are significantly different ($P<0.05$) by SNK test.

72h when mortality was high, the Infection Index was 0.0. At 24h, there was enough survival to not significantly alter the Infectivity Index of 5B. A similar comparison can be made when diazinon was compared from Fig. 3-3 and Table 3-2. Mortality increases at 120h were coincident with a significant drop in the Infectivity Index at 120h at 1.0 mg/ml concentration.

Infection of *G. mellonella* larvae by L1C juveniles was significantly lower ($P<0.05$) for nematodes treated with chlordane and methoxychlor. Comparison of Fig. 3-4 with Table 3-3 illustrates the relationship between mortality and infection success. Diazinon and carbaryl (Fig. 3-5), increased mortality at 1.0 mg/ml, but did not significantly affect the capacity of juveniles to infect *G. mellonella*. The other insecticides showed no significant effect on Infection Index of 5B nematodes.

Chlordane showed no sublethal effects to L1C nematodes at concentrations $\leq$0.1 mg/ml. Likewise, methoxychlor did not show significant effects on Infection Index at concentrations $\leq$0.1 mg/ml (Table 3-4). Both these chemicals were toxic to L1C infective juveniles at 1.0 mg/ml (Fig. 3-4) and as a result, the Infection Index was significantly lower, as most nematodes were killed by the chemical.

3.2. TOXICITY OF INSECTICIDES TO IN VITRO REPRODUCTION

Although adult nematodes were counted along with juveniles, the total was frequently low; accurate estimation of the adult population after the dilution procedure was suspect as small differences in counts in a dilution contributed to large differences in adult population estimation. Nematodes were scored from 1-7 based on the number of juveniles (Table 3-5).

Table 3-3: Recovery of *Steinernema* sp. Newfoundland strain no. 1 (L1C) after exposure to insecticides and subsequent infection in *G. mellonella*.[+]

	% *G. mellonella* with nematodes after exposure(h) to insecticides							
	Organochlorines							
Concentration	Chlordane				Methoxychlor			
(mg/ml)	24	72	120	168	24	72	120	168
0.0	100	100	100	100	100	100	100	100
0.001	100	100	100	100	100	100	100	100
0.01	100	100	100	100	100	100	100	100
0.1	100	100	100	100	100	100	100	100
1.0	8*	0*	13*	0*	0*	0*	0*	0*
	(11.5)		(23.1)		(11.5)			

	Organophosphates			
	Malathion	Diazinon		
	24-168	24	72-120	168
0.0	100	100	100	100
0.001	100	100	100	100
0.01	100	100	100	100
0.1	100	100	100	100
1.0	100	93	100	80
		(11.5)		(34.6)

	Carbamates		Insect Growth Regulator
	Carbaryl	Matacil	Dimilin
	24-168	24-168	24-168
0.0	100	100	100
0.001	100	100	100
0.01	100	100	100
0.1	100	100	100
1.0	100	100	100
0.0(S.C.)[++]	-	-	100

[+]Combined data of three replicates with five larvae per replicate. Standard deviations are bracketed. Standard deviations were ±0.0 unless indicated.

[++]Solvent-treated control (1% v/v).

*Significantly different ($P<0.05$) from control by SNK test.

Table 3-4: Recovery of *Steinernema* sp. Newfoundland strain no. 1 (L1C) from *G. mellonella* by dissection after exposure to selected insecticides. Sample statistics are mean ± SD.[+]

Insecticide Conc. (mg/ml)	Mean No. Nematodes per Larvae[++] 24h	72h	120h	168h
Methoxychlor				
1.0	1.0 (1) aA	0.0 (0) aA	0.0 (0) aA	0.0 (0) aA
0.1	6.9 ± 1.9(15) aB	7.2 ± 2.2(15) aB	7.4 ± 2.3(15) aB	8.1 ± 3.1(15) aB
0.01	8.5 ± 3.2(15) aB	7.7 ± 2.2(15) abB	6.1 ± 1.6(15) bB	8.7 ± 3.3(15) aB
0.001	6.1 ± 2.2(15) aB	6.0 ± 2.0(15) aB	6.4 ± 3.2(15) aB	9.3 ± 3.2(15) bB
0.0	6.9 ± 3.2(15) aB	7.3 ± 2.1(15) aB	6.9 ± 2.9(15) aB	8.6 ± 2.3(15) aB
Diazinon				
1.0	4.3 ± 2.2(14) aA	7.1 ± 2.6(15) bA	6.3 ± 2.7(15) bA	3.0 ± 2.1(12) aA
0.1	5.2 ± 1.4(15) aAB	7.3 ± 2.5(15) bA	7.3 ± 2.1(15) bA	6.6 ± 1.2(15) bB
0.01	4.9 ± 2.1(15) aAB	7.3 ± 1.9(15) bA	6.5 ± 3.0(15) abA	8.2 ± 3.1(15) bBC
0.001	5.9 ± 1.7(15) aAB	8.1 ± 1.8(15) bA	6.2 ± 2.5(15) aA	6.3 ± 2.0(15) aB
0.0	6.9 ± 3.2(15) aB	7.3 ± 2.1(15) aA	6.9 ± 2.9(15) aA	8.6 ± 2.3(15) aC
Chlordane				
1.0	1.0 (1) aA	0.0 (0) aA	1.0 ± 0.0 (2) aA	0.0 (0) aA
0.1	8.0 ± 2.1(15) aB	7.1 ± 2.5(15) aB	7.4 ± 1.3(15) aB	8.9 ± 2.7(15) aB
0.01	7.3 ± 2.6(15) aB	7.4 ± 1.4(15) aB	6.1 ± 1.7(15) aB	7.9 ± 3.4(15) aB
0.001	7.3 ± 2.7(15) aB	7.1 ± 2.7(15) aB	6.3 ± 2.3(15) aB	8.5 ± 3.2(15) aB
0.0	6.9 ± 3.2(15) aB	7.3 ± 2.1(15) aB	6.9 ± 2.9(15) aB	8.6 ± 2.3(15) aB

[+]Sample size is bracketed

[++]Means followed by different lowercase letters in a row and by different uppercase letters in a column are significantly different ($P<0.05$) by SNK test.

Table 3-5: Population scores of nematodes based on the number of juveniles at 14 days post-inoculation.

Score**	Number of Nematodes* Juveniles
1	≥0+
2	>100
3	>500
4	>1000
5	>5000
6	>10,000
7	>50,000

*Number of juveniles in 10 ml of Ringer solution after harvesting from LB medium.

**Adapted from Hara and Kaya (1983a).

+Initial inoculum.

The organophosphate insecticides inhibited reproductive development of 5B. Both malathion and diazinon were toxic at $\geq$0.001 mg/ml (Table 3-6). Methoxychlor was significantly toxic ($P<0.05$) at 1.0 mg/ml while the other organochlorine, chlordane, was toxic at $\geq$0.01 mg/ml. Matacil was toxic to 5B at $\geq$0.001 mg/ml. Concentrations of 1.0 and 0.1 were more toxic than 0.01 and 0.001 mg/ml. Control, DMSO control (1% v/v) and 0.001 mg/ml of Dimilin were not significantly different.

Table 3-7 indicates the toxicity of all insecticides to L1C nematode reproduction. Both diazinon and malathion were significantly toxic at $\geq$0.01 mg/ml. L1C was susceptible to methoxychlor at $\geq$0.1 mg/ml, while chlordane was toxic at $\geq$0.01 mg/ml. Carbaryl was toxic to L1C at 0.1 and 1.0 mg/ml. The other carbamate insecticide, Matacil, was toxic at 0.1 mg/ml and 1.0 mg/ml. However, toxicity at 0.01 mg/ml was not significantly different from control growth but was more lethal to nematode development than exposure to 0.001 mg/ml. The action of the insect growth regulator, although toxic at 0.01 and 1.0 mg/ml, was low relative to the other six insecticides.

3.3. EFFECT OF INSECTICIDE ON SYMBIOTIC BACTERIA

3.3.1. Effect on Generation Time

The generation or doubling time of *Xenorhabdus* sp. isolate from 5B at 15°C was found to be 116.6 $\pm$ 7.2 minutes (Table 3-8). Both organochlorine insecticides at the concentrations tested (0.01, 0.001 mg/ml) did not significantly affect generation time. The organophosphate insecticide, malathion, significantly

Table 3-6: Effect of insecticides on *in vitro* development of *Steinernema* sp. Newfoundland strain no. 2 (5B) juvenile nematodes. Sample statistics are mean population scores ± SD (n=18).

Concentration (mg/ml)	Mean Population Scores*	
	Organochlorines	
	Chlordane	Methoxychlor
0.0	4.4 ± 0.8 A	4.4 ± 0.8 A
0.001	3.6 ± 0.9 A	3.9 ± 1.1 A
0.01	1.0 ± 0.0 B	4.1 ± 0.2 A
0.1	1.3 ± 0.7 B	3.6 ± 1.1 A
1.0	1.0 ± 0.0 B	1.0 ± 0.0 B
	Organophosphates	
	Malathion	Diazinon
0.0	4.4 ± 0.8 A	4.4 ± 0.8 A
0.001	2.2 ± 1.3 B	1.3 ± 0.8 B
0.01	2.2 ± 1.3 B	1.0 ± 0.0 B
0.1	1.0 ± 0.0 B	1.0 ± 0.0 B
1.0	1.0 ± 0.0 B	1.0 ± 0.0 B
	Carbamates	
	Carbaryl	Matacil
0.0	4.4 ± 0.8 A	4.4 ± 0.8 A
0.001	3.5 ± 0.9 AB	2.8 ± 1.2 B
0.01	2.2 ± 1.2 BC	2.7 ± 1.4 B
0.1	1.3 ± 0.5 C	1.0 ± 0.0 C
1.0	1.1 ± 0.2 C	1.0 ± 0.0 C
	Insect Growth Regulator	
	Dimilin	
0.0(S.C.)+	4.0 ± 0.6 A	
0.0	4.4 ± 0.8 A	
0.001	3.5 ± 1.0 AB	
0.01	2.9 ± 1.3 BC	
0.1	2.1 ± 1.3 CD	
1.0	1.2 ± 0.5 D	

*All scores followed by different uppercase letters in a column were significantly different ($P<0.05$) by nonparametric multiple comparisons procedure.

+Solvent-treated control (1% v/v).

Table 3-7: Effect of insecticides on *in vitro* development of *Steinernema* sp. Newfoundland strain no. 1 (L1C) juvenile nematodes. Sample statistics are mean population scores ± SD (n=18).

Concentration (mg/ml)	Mean Population Scores*	
	Organochlorines	
	Chlordane	Methoxychlor
0.0	4.1 ± 1.2 A	4.1 ± 1.2 A
0.001	3.4 ± 1.0 AB	3.3 ± 1.7 AB
0.01	2.1 ± 1.3 BC	3.9 ± 1.9 A
0.1	1.9 ± 0.9 C	1.9 ± 1.5 B
1.0	1.0 ± 0.0 C	1.0 ± 0.0 C
	Organophosphates	
	Malathion	Diazinon
0.0	4.1 ± 1.2 A	4.1 ± 1.2 A
0.001	2.5 ± 1.2 AB	4.1 ± 1.3 A
0.01	2.0 ± 1.1 BC	1.0 ± 0.0 B
0.1	1.0 ± 0.0 C	1.0 ± 0.0 B
1.0	1.0 ± 0.0 C	1.0 ± 0.0 B
	Carbamates	
	Carbaryl	Matacil
0.0	4.1 ± 1.2 A	4.1 ± 1.2 AB
0.001	3.4 ± 0.7 A	5.1 ± 0.9 A
0.01	3.7 ± 1.5 A	2.7 ± 1.1 B
0.1	1.1 ± 0.5 B	1.1 ± 0.3 C
1.0	1.0 ± 0.0 B	1.0 ± 0.0 C
	Insect Growth Regulator	
	Dimilin	
0.0(S.C.)+	4.0 ± 0.0 A	
0.0	4.1 ± 1.2 A	
0.001	3.9 ± 1.8 A	
0.01	2.4 ± 1.4 B	
0.1	3.2 ± 1.5 AB	
1.0	2.0 ± 1.1 B	

*All scores followed by different uppercase letters in a column were significantly different ($P<0.05$) by nonparametric multiple comparisons.

+Solvent-treated control (1% v/v).

increased generation time at 0.1 mg/ml and diazinon also increased generation time at 0.01 mg/ml. The carbamate insecticide, Matacil, did not significantly affect generation time while carbaryl caused an increase in the doubling time at 0.01 mg/ml. The insect growth regulator Dimilin significantly lowered generation time at 0.001 mg/ml. This effect was likely due to DMSO as the solvent control (DMSO 1% v/v) was significantly lower than baseline generation time.

The generation time of *Xenorhabdus* sp. isolate from L1C at 15°C was 90.2 $\pm$ 5.9 minutes (Table 3-9). Chlordane increased generation time at 0.01 mg/ml while the other organochlorine did not affect generation time at 0.01 and 0.001 mg/ml. Malathion increased generation time at 0.1 mg/ml, while diazinon significantly increased ($P<0.05$) generation time at 0.01 and 0.001 mg/ml. Both carbamates increased the doubling time of L1C bacteria at both test concentrations. Dimilin exerted a toxic effect on generation time at 0.01 and 0.001 mg/ml. DMSO did not significantly alter generation time of the L1C bacterial symbiont.

3.3.2. Insecticidal Effect on Growth on Solid Media

The organochlorine insecticides affected low cell numbers (<300) of both the *Xenorhabdus* sp. from L1C and *Xenorhabdus* sp. isolate from 5B at 1.0 mg/ml. The L1C isolate was sensitive to both organochlorines at 0.1 mg/ml, whereas the 5B bacteria was not (Table 3-10 and Table 3-11). Methoxychlor affected low numbers of bacterial isolate L1C over all tested concentrations. However, enough cells from a thick cell suspension of either bacterial isolate, survived to form a confluent lawn of growth on plates treated with either chlordane or methoxychlor at $\leq$ 1.0 mg/ml (Table 3-12).

Table 3-8: Generation times of *Xenorhabdus* sp. (5B) in broth medium[+] containing insecticide. Sample statistics are mean ± SD (n=4).

Insecticide	Insecticide Concentration (mg/ml) 0.1	0.01	0.001	0.0
Chlordane	-	109.7 (5.6)[**]	111.6 (7.4)	116.6 (7.2)
Methoxychlor	-	113.2 (5.3)	110.4 (0.9)	116.6 (7.2)
Malathion	251.4[*] (142.1)	107.1 (1.3)	110.4 (5.6)	116.6 (7.2)
Diazinon	-	137.2[*] (5.4)	112.8 (3.8)	116.6 (7.2)
Carbaryl	-	138.2[*] (16.5)	117.7 (5.6)	116.6 (7.2)
Matacil	-	109.8 (10.3)	100.4 (7.2)	116.6 (7.2)
Dimilin	-	122.9 (2.5)	108.5[*] (2.5)	116.6 (7.2)
S.C.[++]	-	-	-	107.5[*] (2.8)

[+]Growth medium contains 1.6% nutrient broth and 0.2% yeast extract incubated at 15°C.

[++]Solvent-treated control (1%v/v).

[*]Represents mean significantly different (P<0.05) from control by SNK test.

[**]Bracketed values are ± standard deviation.

Table 3-9: Generation times of *Xenorhabdus* sp. (L1C) in broth medium[+] containing insecticide. Sample statistics are mean ± SD (n=4).

Insecticide	Insecticide Concentration (mg/ml)			
	0 .1	0.01	0.001	0.0
Chlordane	-	135.7* (18.8)[++]	88.8 (4.1)	90.2 (5.9)
Methoxychlor	-	97.8 (4.5)	100.1 (9.2)	90.2 (5.9)
Malathion	121.9* (2.6)	90.5 (2.1)	97.6 (7.7)	90.2 (5.9)
Diazinon	-	157.1* (30.3)	106.3* (8.6)	90.2 (5.9)
Carbaryl	-	82.2* (1.8)	80.0* (1.7)	90.2 (5.9)
Matacil	-	137.9* (15.7)	104.4* (2.6)	90.2 (5.9)
Dimilin	-	102.4* (6.3)	127.9* (11.1)	90.2 (5.9)
S.C.[++]	-	-	-	97.9 (4.4)

[+]Growth medium contains 1.6% nutrient broth and 0.2% yeast extract incubated at 15°C.

[++]Solvent-treated control (1% v/v).

*Represents means significantly different ($P<0.05$) from control by SNK test.

[++]Bracketed values are ± standard deviation.

Table 3-10: Survival of *Xenorhabdus* sp. (5B) on insecticide-treated media 6 days after initial exposure.*

Concentration (mg/ml)	Mean % Survival + SD (n=2)		
	Carbaryl	Matacil	Dimilin+
1.0	0.0 ± 0.0	0.0 ± 0.0	58.3 ± 17.3
0.1	0.0 ± 0.0	0.0 ± 0.0	61.5 ± 0.2
0.01	100.0 ± 0.0	0.0 ± 0.0	53.5 ± 10.2
0.001	99.7 ± 0.3	0.0 ± 0.0	44.6 ± 8.3
	Chlordane	Methoxychlor	
1.0	0.0 ± 0.0	0.0 ± 0.0	
0.1	9.0 ± 12.7	94.7 ± 7.4	
0.01	82.1 ± 25.2	100.0 ± 0.0	
0.001	100.0 ± 0.0	83.9 ± 22.8	
	Diazinon	Malathion	
1.0	0.0 ± 0.0	1.5 ± 0.2	
0.1	0.0 ± 0.0	94.3 ± 8.0	
0.01	100.0 ± 0.0	100.0 ± 0.0	
0.001	100.0 ± 0.0	100.0 ± 0.0	

*Nutrient broth (1.6%), yeast extract (0.2%), agar (1.5%), incubation at 15°C.

+100 % survival of bacteria on solvent-treated (DMSO) control (1 % v/v).

Table 3-11: Survival of *Xenorhabdus* sp. (L1C) on insecticide-treated media 6 days after initial exposure.*

Concentration (mg/ml)	Mean % Survival + SD (n=2)		
	Carbaryl	Matacil	Dimilin
1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.01	15.5 ± 8.3	0.0 ± 0.0	4.7 ± 4.9
0.001	33.9 ± 32.7	7.0 ± 5.9	17.4 ± 21.8
	Chlordane	Methoxychlor	
1.0	0.0 ± 0.0	0.0 ± 0.0	
0.1	1.5 ± 2.2	1.9 ± 1.6	
0.01	17.0 ± 0.0	21.2 ± 22.0	
0.001	100.0 ± 0.0	12.5 ± 12.6	
	Diazinon	Malathion	
1.0	0.0 ± 0.0	0.0 ± 0.0	
0.1	5.7 ± 4.0	27.5 ± 23.1	
0.01	100.0 ± 0.0	100.0 ± 0.0	
0.001	100.0 ± 0.0	98.0 ± 0.0	

*Nutrient broth (1.6%), yeast extract (0.2%), agar (1.5%), incubation at 15°C.

+100 % survival of bacteria on solvent-treated (DMSO) control (1% v/v).

Table 3-12: Minimum insecticide concentration failing to produce visible colonies on insecticide-treated nutrient plates.[+]

Insecticide Class	Insecticide	Minimum Inhibitory Concentration (mg/ml) Species L1C	5B
Organochlorine	Chlordane	>1.0*	>1.0
	Methoxychlor	>1.0	>1.0
Organophosphate	Malathion	>1.0	>1.0
	Diazinon	1.0	1.0
Carbamate	Carbaryl	0.1[++]	0.1
	Matacil	1.0	>1.0
Insect Growth Regulator	Dimilin	>1.0	>1.0
	S.C.**	>1.0	>1.0

[+]A thick cell suspension, similar to that used for *in vitro* nematode growth experiments, was inoculated on nutrient broth (1.6%), yeast extract (0.2%), agar (1.5%); incubation at 15°C for 6 days.

[++]Six replicate plates per chemical were tested.

*The minimum inhibitory concentration was above the upper practical concentration of insecticide utilized in experimental procedures.

**Solvent treated control (1% v/v).

3.4. MIGRATION OF NEMATODES IN SOIL

3.4.1. Effect of *G. mellonella* larvae on Migration

The presence of ten *G. mellonella* larvae at the base of a soil column significantly ($P<0.05$) affected the degree of migration of both L1C and 5B infective juveniles. Table 3-13 shows that the percentage of inoculated nematodes reaching the 8-10 cm depth in the cylinder was significantly different ($P<0.05$) in cylinders containing *G. mellonella* (8.9%) from those in cylinders without *G. mellonella* larvae (0.0%). Likewise, L1C nematodes migrated in significant numbers in cylinders with larvae (2.7%) as opposed to cylinders without larvae (0.1%) (Table 3-14). Larger numbers of nematodes migrated from the top layer in cylinders with larvae at the base.

Although the magnitude of migration in the middle layers (2-8 cm) of the cylinders was not significantly different there was a trend towards more downward migration in cylinders with *G. mellonella* larvae for both L1C and 5B nematodes.

Recovery of nematodes between cylinders with and without *G. mellonella* were not significantly different within species (Table 3-15). L1C recovery in the absence of *G. mellonella* was significantly more than 5B nematodes

Table 3-13: Vertical distribution of *Steinernema* sp. Newfoundland strain no. 2 (5B) infective juveniles in the presence and absence of ten *G. mellonella* larvae located at the base of a 10 cm soil column 48h after placement of 3000 nematodes in the top soil layer.

	Mean No. Juveniles ± SD (n=10)*	
Depth (cm)	P+	A+
0-2	1684.2 ± 173.8 Aa	2290.2 ± 140.0 Aa
2-4	489.3 ± 150.8 Ba	497.2 ± 107.8 Ba
4-6	286.3 ± 71.3 Ca	158.4 ± 72.7 Ca
6-8	271.4 ± 83.4 Ca	53.3 ± 71.5 Da
8-10	268.8 ± 85.6 Ca	0.9 ± 2.0 Eb

*Means followed by different uppercase letters in a column and different lowercase letters in a row represent significant differences (P<0.05) by SNK test.

+P= *G. mellonella* larvae present at 8-10 cm depth. A= *G. mellonella* larvae absent.

Table 3-14: Vertical distribution of *Steinernema* sp. Newfoundland strain no. 1 (L1C) infective juveniles in the presence and absence of ten *G. mellonella* larvae located at the base of a 10 cm soil column 48h after placement of 3000 nematodes in the top soil layer.

	Mean No. Juveniles ± SD (n=10)*	
Depth (cm)	P+	A+
0-2	1807.8 ± 333.0 Aa	2431.7 ± 107.2 Ab
2-4	736.1 ± 240.9 Ba	485.7 ± 166.1 Ba
4-6	258.0 ± 116.8 Ca	68.0 ± 45.3 Ca
6-8	117.3 ± 52.4 Da	12.1 ± 10.3 Da
8-10	80.8 ± 48.9 Da	2.5 ± 3.1 Db

*Means followed by different uppercase letters in a column and different lowercase letters in a row represent significant differences (P<0.05) by SNK test.

+P= *G. mellonella* larvae present at 8-10 cm depth. A= *G. mellonella* larvae absent.

Table 3-15: Recovery of infective juvenile nematodes 48h after inoculation of 3000 nematodes in cylinders with and without ten *G. mellonella* larvae located at the base.

	Mean % Recovery ±SD (n=5)	
Species	P*	A*
L1C	37.4 + 6.5 a+	35.5 + 13.3 a
5B	35.4 + 4.8 ab	29.7 + 4.3 b

*P= *G. mellonella* larvae present at 8-10 cm depth. A= (G. mellonella) larvae absent.

+Means followed by different lowercase letters represent significant differences (P<0.05) by Students T-test.

3.4.2. Effect of Insecticides on Migration

During this series of experimnets, nematodes were recovered from soil by the Baermann funnel technique. Recovery in this study was usually 30-40% of the original inoculum and therefore, values were converted to a proportion of the original inoculum. It was assumed that recovery efficiency throughout individual layers of soil from a single column were equal.

The organochlorine insecticide chlordane, had no significant ($P<0.05$) effect on migration of L1C or 5B over the range of concentrations tested (Table 3-16 and Table 3-17).

The organophosphate insecticide diazinon significantly affected migration of 5B juvenile nematodes. Migration at $\leq$ 0.01 mg/ml was unaffected, but at 0.1 mg/ml diazinon, migration was significantly affected as the number of migrating nematodes declined at the 4-6 cm level and significantly more 5B juveniles remained in the initial layer of soil for both 0.1 and 1.0 mg/ml concentrations (Table 3-18).

Diazinon had a similar effect on L1C. At $\leq$ 0.01 mg/ml, migration was not significantly altered. A larger proportion of nematodes remained in the initial (0-2 cm) layer at 1.0 mg/ml and differences in downward migration could be detected in all layers. At 0.1 mg/ml, migrational difference became significant at the 4-6 cm level (Table 3-19).

Table 3-20 shows that recovery of L1C nematodes at 1.0 mg/ml chlordane and 5B nematodes at $\geq$ 0.1 mg/ml was significantly different from the control.

Table 3-16: Vertical distribution of *Steinernema* sp. Newfoundland strain no. 2 (5B) infective juveniles in chlordane-treated soil columns 48 hours after inoculation of 3000 nematodes in top soil layer.

	Mean No. Juveniles ±SD (n=5)*									
Depth (cm)	Concentration (mg/ml) 0.0		0.001		0.01		0.1		1.0	
0-2	1638.8 (±160.0)	Aa	1601.0 (±340.4)	Aa	1830.2 (±291.3)	Aa	1886.0 (±261.5)	Aa	1841.2 (±353.3)	Aa
2-4	483.8 (±197.0)	Ba	484.4 (±170.6)	Ba	461.0 (±95.2)	Ba	477.2 (±56.9)	Ba	455.0 (±174.9)	Ba
4-6	329.4 (±41.3)	BCa	249.8 (±101.5)	Ba	246.6 (±115.7)	Ca	241.2 (±29.7)	Ca	265.0 (±103.5)	BCa
6-8	283.0 (±78.8)	Ca	390.8 (±146.9)	Ba	197.4 (±78.6)	Ca	222.2 (±80.9)	Ca	276.0 (±104.4)	BCa
8-10	265.0 (±117.4)	Ca	274.0 (±111.1)	Ba	264.8 (±142.9)	Ca	173.4 (±114.6)	Ca	162.2 (±47.5)	Ca

++Means followed by different lowercase letters in a row and by different uppercase letters in a column are significantly different ($P<0.05$) by SNK test. Standard deviation is bracketed.

Table 3-17: Vertical distribution of *Steinernema* sp. Newfoundland strain no. 1 (L1C) infective juveniles in chlordane-treated soil columns 48 hours after inoculation of 3000 nematodes in top soil layer.

	Mean No. Juveniles ±SD (n=5)*									
Depth (cm)	Concentration (mg/ml) 0.0		0.001		0.01		0.1		1.0	
0-2	1812.2 (±293.7)	Aa	1916.4 (±249.0)	Aa	2129.8 (±218.2)	Aa	2175.8 (±260.1)	Aa	2169.4 (±250.5)	Aa
2-4	728.6 (±235.0)	Ba	603.0 (±44.6)	Ba	521.8 (±123.5)	Ba	495.0 (±110.6)	Ba	489.2 (±86.3)	Ba
4-6	281.4 (±134.7)	Ca	212.4 (±91.0)	Ca	175.4 (±49.8)	Ca	166.6 (±69.3)	Ca	161.0 (±67.7)	Ca
6-8	105.6 (±41.6)	Da	160.0 (±90.9)	Ca	97.4 (±31.8)	Da	81.8 (±29.3)	Ca	86.8 (±38.6)	Ca
8-10	72.2 (±42.4)	Da	108.2 (±58.9)	Ca	75.6 (±40.4)	Da	80.8 (±76.0)	Ca	93.6 (±100.1)	Ca

*Means followed by different lowercase letters in a row and by different uppercase letters in a column are significantly different ($P<0.05$) by SNK test. Standard deviation is bracketed.

Table 3-18: Vertical distribution of *Steinernema* sp. Newfoundland strain no. 2 (5B) infective juveniles in diazinon-treated soil columns 48 hours after inoculation of 3000 nematodes in top soil layer.

	Mean No. Juveniles ±SD (n=5)*									
Depth (cm)	Concentration (mg/ml) 0.0		0.001		0.01		0.1		1.0	
0-2	1638.8 (±160.0)	Aa	1232.8 (±309.5)	Ab	1662.2 (±339.9)	Aac	2193.2 (±287.0)	Ad	2464.6 (±237.9)	Ad
2-4	483.8 (±197.0)	Ba	496.0 (±124.3)	Ba	415.2 (±83.2)	Ba	411.6 (±145.2)	Ba	475.6 (±205.7)	Ba
4-6	329.4 (±41.3)	BCa	346.0 (±90.3)	Ba	274.6 (±36.2)	Ba	202.8 (±92.7)	Ca	55.6 (±65.8)	Cb
6-8	283.0 (±78.8)	Ca	538.6 (±173.8)	Bb	363.8 (±158.9)	Ba	128.0 (±59.5)	CDc	11.2 (±9.4)	Dd
8-10	265.0 (±117.4)	Ca	386.6 (±135.3)	Ba	284.2 (±156.4)	Ba	64.4 (±42.4)	Db	0.0 (±0.0)	Dc

**Means followed by different lowercase letters in a row and by different uppercase letters in a column are significantly different ($P<0.05$) by SNK test. Standard deviation is bracketed.

Table 3-19: Vertical distribution of *Steinernema* sp. Newfoundland strain no. 1 (L1C) infective juveniles in diazinon-treated soil columns 48 hours after inoculation of 3000 nematodes in top soil layer.

	Mean No. Juveniles ±SD (n=5)*									
Depth (cm)	Concentration (mg/ml) 0.0		0.001		0.01		0.1		1.0	
0-2	1812.2 (±293.7)	Aa	1953.0 (±196.4)	Aa	1764.4 (±405.5)	Aa	2244.0 (±238.5)	Aa	2725.0 (±149.0)	Aa
2-4	728.6 (±235.0)	Ba	667.0 (±58.8)	Ba	653.4 (±102.3)	Ba	562.0 (±127.5)	Ba	237.2 (±135.9)	Bb
4-6	281.4 (±134.7)	Ca	205.6 (±60.8)	Cab	286.8 (±119.1)	CDab	122.0 (±79.3)	Cb	35.0 (±32.2)	Cc
6-8	105.6 (±41.6)	Dab	116.6 (±85.5)	Dab	191.6 (±149.9)	DEa	49.2 (±43.0)	Db	2.8 (±6.3)	Cc
8-10	72.2 (±42.4)	Da	57.8 (±24.3)	Da	103.8 (±71.2)	Ea	22.8 (±29.1)	Db	0.0 (±0.0)	Cb

++Means followed by different lowercase letters in a row and by different uppercase letters in a column are significantly different ($P<0.05$) by SNK test. Standard deviation is bracketed.

Although recovery was affected by the higher concentrations of chlordane, the migration rate was not affected even at 1.0 mg/ml.

Diazinon significantly affected recovery of 5B at 1.0 mg/ml, despite low toxicity at 72h (14.7%) (Fig. 3-3). Toxicity on L1C was low at 72h (6.7%) (Fig. 3-5) and in fact recovery was unaffected. Despite recovery being unaffected, the migration of nematodes was clearly altered at $\geq$ 0.1 mg/ml for both nematode species (Table 3-18 and Table 3-19) in the presence of diazinon.

Table 3-20: Recovery of infective juveniles 48h after inoculation of 3000 nematodes in insecticide treated cylinders.

Species	Insecticide Conc. (mg/ml)	Mean % Recovery ± SD (n=5)[+] Chlordane	Diazinon
L1C	1.0	23.0 ± 3.9*	25.1 ± 6.0
	0.1	31.1 ± 2.8	38.6 ± 6.6
	0.01	37.6 ± 12.0	37.8 ± 8.3
	0.001	32.8 ± 5.0	38.1 ± 15.5
	0.0	38.5 ± 5.5	38.5 ± 5.5
5B	1.0	15.6 ± 1.3*	15.3 ± 6.5*
	0.1	25.1 ± 5.1*	27.8 ± 7.0
	0.01	27.9 ± 5.0	27.3 ± 11.0
	0.001	31.9 ± 8.9	36.9 ± 8.9
	0.0	35.6 ± 4.4	35.6 ± 4.4

[+]Means followed by an asterisk are significantly different ($P<0.05$) from control by SNK test.

Chapter 4

DISCUSSION

Unimodal pest control strategies have been successful in reducing pest populations below economic injury thresholds. Insecticide use has generally been successful but has come under scrutiny from elevated public awareness and concern over adverse effects on the ecosystem, as well as target organism resistance and increasingly higher costs of manufacturing selective insecticides. Biological control provides an effective and environmentally acceptable alternative to chemical control methods (Burman *et al.* 1979; Poinar 1986).

The efficiency and relative low cost of wide-spectrum insecticides cannot be ignored and their use is likely to continue for some time. The integration of entomogenous nematodes with an insecticide program may make it possible to lower the volume of insecticide used. This reduces the levels of insecticide in the environment and other deleterious effects on the ecosystem, while not compromising agricultural and forest yields. This study examined the compatability of several insecticides with steinernematid nematodes, potentially useful biological control agents (Poinar 1986).

4.1. DIRECT EXPOSURE TO INSECTICIDES BY IMMERSION

Immersion of juveniles in aqueous suspensions of insecticide served to establish the protective effect of the double cuticle in the free-living infective stage of the steinernematid nematodes. While some insecticide concentrations caused mortality, exposure to lower concentrations resulted in decreased motility and paralysis. Prakasa Rao *et al.* (1975) found several organophosphates, such as monocrotophos, phosalone and fenitrothion, directly toxic to *S. dutkyi* at $\geq$0.5 mg/ml, while vanidothion was not toxic. Hara and Kaya (1983a) found phenamiphos toxic to *S. feltiae* at 0.5 mg/ml. In this study, when infective juveniles of both L1C and 5B were directly exposed to diazinon at 1.0 mg/ml, substantial (ca. 50%) mortality occurred after 120h of exposure. There appear to be variations in sensitivity of steinernematid species to different organophosphate insecticides. This should be considered in any integrated pest management program.

Fedorko *et al.* (1977a,1977b) found the carbamate insecticides, dioxacarb and oxamyl, toxic to *S. feltiae* (LC_{50} > or 1.0 mg/ml), but there was no mortality at concentrations below 1.0 mg/ml. Hara and Kaya (1983a) noted little direct mortality after exposure to 1.0 mg/ml methomyl after 168h of exposure. During this study, the carbamates, carbaryl and Matacil (aminocarb) were assayed and while Matacil had no observable toxic effect on either nematode, carbaryl caused low (<25%) mortality of L1C at 1.0 mg/ml (1000 ppm), a finding in general agreement with previous reports. The Matacil assayed was formulated in oil and

thus did not mix well with the water suspension of nematodes; as a result contact was limited and likely affected these results. The two strains of *Steinernema* examined in this study resemble *S. feltiae* in their tolerance to the insect growth regulator, Dimilin (Hara and Kaya 1983a).

In separate studies by Fedorko *et al.* (1977a) and Hara and Kaya (1983a), the organochlorine insecticide methoxychlor, was not toxic to *S. feltiae* at $\leq$1.0 mg/ml. However, both L1C and 5B juvenile nematodes were found to be sensitive to 1.0 mg/ml of methoxychlor and chlordane. It should be noted that although there was low 5B mortality after 24h, a portion of the sampled population was affected by chlordane and by 72h substantial mortality was observed. Differences noted for methoxychlor may be attributed to species or strain differences. In addition, commercially formulated methoxychlor was used in the present study, as opposed to the technical product used by previous researchers, this may account for the difference in results.

Nematodes in the affected state, which was characterized by slow movement and delayed response to probing, were only seen in significant proportions ($>$50%) after exposure to diazinon and chlordane at 1.0 mg/ml for 5B nematode. Although this state was seen for other chemicals and L1C, it infrequently exceeded 5% of the sampled population. Hara and Kaya (1983a) consistently observed this affected condition, which they termed 'sublethal effect', after exposure of *S. feltiae* juveniles to the organophosphates, methomyl ($\geq$0.1 mg/ml) and phenamiphos ($\geq$0.01 mg/ml). They did not observe lethal or sublethal affects for juveniles exposed to the organochlorine, methoxychlor. According to

Keetch (1974), nematodes in this condition progress to death unless the insecticide is removed by washing; subsequently nematode infectivity would be restored (Hara and Kaya 1983a). The progression from the affected state to death was evident during exposure of 5B to 1.0 mg/ml diazinon. At 24h the percentage of dead nematodes was 24% of the population and increased to 50% over the course of the experiment. The reason why nematodes were not observed in the sublethal state, prior to mortality after exposure to other insecticides, is difficult to explain. In some cases, the time course from alive to sublethal to a lethal effect may be short if the insecticide is particularly toxic (eg. 1.0 mg/ml chlordane at 24h).

The paralysis and eventual death of both L1C and 5B infective juveniles occurred after exposure to 1.0 mg/ml of both organochlorine insecticides, chlordane and methoxychlor. The organophosphate, diazinon was toxic at 1.0 mg/ml for both nematodes. A significantly greater number of 5B nematodes were sublethally affected by 1.0 mg/ml diazinon. It was likely that the cuticle provided some protection which eventually broke down over time. This may be due to rates of penetration, metabolism and detoxification of the chemical by the nematode. No observable toxic effects were seen for Dimilin, malathion and Matacil at non-lethal concentrations.

4.2. IMPACT OF DIRECT INSECTICIDE EXPOSURE ON INFECTION INDEX

Recovery of adult nematodes from moribund *G. mellonella* larvae, after infection with juveniles previously exposed to insecticide, was unaffected for both L1C and 5B after exposure to Dimilin, malathion and both carbamates. This

result was not unexpected as significant toxicity was not observed at any of the tested concentrations. However, diazinon, methoxychlor and chlordane exerted observable toxic effects at 1.0 mg/ml in previous experiments so that in addition to examining the effect of these insecticides on infection success, the Infection Index was also assessed.

Diazinon at 1.0 mg/ml was progressively more toxic to both L1C and 5B as exposure time increased. Infection Index dropped concomitantly as lethal effect increased, yet it was unclear if this decrease in Infectivity Index was due to sublethal effects on remaining nematodes or the value was simply a reflection of the decline in the number of living nematodes.

The lethal effect of methoxychlor and chlordane on L1C at 1.0 mg/ml resulted in no infection of *G. mellonella*. Likewise, 5B mortality was high after exposure to 1.0 mg/ml methoxychlor and accordingly, Infection Index low. Toxicity of chlordane to 5B was less than toxicity to the L1C strain, however. After 24h of exposure of 5B to 1.0 mg/ml chlordane, over 70% of the nematodes were affected by the insecticide, yet these nematodes were able to infect insect larvae. The Infection Index for either 5B or L1C was unaffected at $\leq$0.1 mg/ml.

The Infection Index data displayed a high level of variability among replicates of the same treatment group. The inoculation of 250 nematodes was only an approximation. Nematodes were suspended in their incubation tubes by agitation prior to withdrawal by pipette, however, they may have settled before the required number of nematodes in an aliquot were removed. Attempts were made

to distribute the nematodes evenly over the filter paper/petri dish experimental chamber, however, settling of nematodes in the water column of the pipette may have resulted in uneven distribution. Some Infection Index values were statistically different from controls but clearly, restraint must be shown in interpreting such results. For 5B exposed to 0.1 mg/ml methoxychlor at 120h, the Infection Index was significantly lower than that of controls; at 168h the difference was no longer seen. Rather than representing a difference of actual biological significance, the result was due to one or more of the technical errors noted previously.

4.3. EFFECT OF INSECTICIDES ON IN VITRO-REARED NEMATODES

The *in vitro* growth of entomogenous nematodes varies with species, medium, propagation technique, presence of associated bacterium and initial nematode inoculum. The objective of this study was not to develop a highly efficient *in vitro* rearing procedure but rather to find a medium that supported nematode development and observe the effect of insecticides on the nematode population and its' associated bacteria in an artificial environment. A high protein medium containing extract of liver and beef was developed and found to be suitable for nematode reproduction and development.

Both L1C and 5B infrequently developed on beef-liver nutrient plates to more than 10,000 nematodes per plate after 14 days of incubation from an original inoculum of 100 nematodes. All insecticides tested exerted a toxic effect on the *in vitro* development and reproduction of both L1C and 5B nematodes.

Development and reproduction begin on culture plates following the exsheathment of the outer cuticle. The developmental stages of the nematode lack the extra protection provided by the second cuticular layer of the free-living juvenile stage. The barrier to chemical insecticides is reduced and they can gain access to the internal structures of the nematode.

It was apparent that the shed cuticle had provided an excellent protective device. Direct exposure data revealed that methoxychlor, chlordane and diazinon exerted a lethal effect only at 1.0 mg/ml on infective juveniles, whereas developing nematodes were susceptible to all insecticides at concentrations not previously toxic to infective juveniles after direct exposure. With the exception of Dimilin, the insecticides tested in this study were potential toxins of the nematode neuromuscular system and this was the possible target site which could account for lack of development *in vitro*. The chitin synthetase inhibitor, Dimilin, reduced populations of both L1C and 5B at $\geq$0.1 mg/ml. Chitin synthesis in nematodes is confined to egg development and chitin synthesis inhibitors have been shown to impair nematode development by preventing chitin synthesis in the egg stage (Veech 1978a, 1978b; Calcott and Fatig 1984). Thus, egg development in 5B and L1C may have been the target site for Dimilin toxicity observed during *in vitro* studies.

Hara and Kaya (1982) found several carbamates and organophosphates impaired development; the impairment was low compared to results of the current experiment. These authors determined that methoxychlor did not affect development, while this study shows both organochlorine insecticides tested were

extremely toxic to development at $\geq$0.01 mg/ml. Differences may have occurred due to different species and use of formulated rather than technical grade insecticides.

Nematode feeding is impaired by carbamate insecticides (Keetch 1974). Steinernematid nematodes feed on bacteria or the products of bacterial metabolism. The feeding ability of the developing and adult nematodes may be impaired by the carbamates or organophosphates by targeting the musculature of the nematodes which is intimately involved with feeding.

4.4. EFFECT OF INSECTICIDE ON SYMBIOTIC BACTERIA

Akhurst (1983) has shown that the primary form of the symbiotic bacteria is necessary for successful *in vitro* growth. Recent studies on the effect of insecticides on *in vitro* reproduction have overlooked the possibility that the chemicals may be exerting a toxic effect on the bacteria in addition to or instead of the nematodes. Insecticides toxic to the bacteria may result in lack of food for nematodes either directly due to death of bacteria or through failure of bacteria to provide metabolic products required by the nematode for growth. Clearly, a study on the effect of insecticide on the growth of the bacterium was necessary.

Any chemical insult to a bacterium may result in slower growth rate and subsequent increase in generation time. By determining the baseline generation time of each isolate in nutrient medium and generation time in the presence of insecticide, the bactericidal contribution to the effect of insecticide on *in vitro* reproduction was determined. Although this situation is experimental and not

likely to occur under natural conditions, it does establish the toxicity of the insecticides to the associated bacterium.

The two bacterial isolates were differentially susceptible to some of the investigated insecticides. For example, Matacil was not toxic at $\leq$0.01 mg/ml for isolate 5B; isolate L1C was very sensitive to the same concentration of this insecticide. The toxicity of the organochlorines and organophosphates to the two strains of bacteria appeared to be similar. The mode of action of bactericidal or bacteriostatic chemicals involves protein denaturation, enzyme inactivation and/or membrane damage, with resultant cell leakage or lysis due to surface-active inhibitory action (Hamilton 1971).

While an insecticide may depress generation time, it does not mean that the bacterium is unable to grow. Since *in vitro* work was carried out on solid agar medium, the effect of the insecticides on the formation of colonies was investigated. This data combined with the direct exposure data aided in assessing whether the reduction during *in vitro* growth of the nematode was due to direct toxicity on the nematode or to adverse effects on the associated bacterium.

Dimilin was not directly toxic to either L1C or 5B infective juveniles, yet *in vitro* growth was significantly reduced at $\geq$0.01 mg/ml. Although Dimilin had some effect on low numbers of both bacteria, the effect on a thick bacterial culture was not seen and thus Dimilin toxicity was directed at egg producing adult nematodes.

Matacil exerted no direct toxic effect on infective 5B or L1C juveniles yet *in*

vitro reproduction was depressed. When a thick cell suspension was applied to Matacil treated plates at 1.0 mg/ml no bacterial lawn grew. At this concentration, it was unclear whether the reduced population resulted from effects on the bacterium or the developing nematodes. Since growth of bacteria on plates treated with $\leq$0.1 mg/ml was confluent or all over the petri dish, *in vitro* toxicity was likely due to toxicity to adult and feeding nematodes.

Both organochlorine insecticides exerted negligible effects on generation time and mass growth of bacteria on plates, but they did inhibit growth of both isolates at $\geq$0.01 mg/ml. Since confluent growth of bacteria was found on plates treated with 1.0 mg/ml, it was unlikely that the bacteria were the limiting factor in *in vitro* nematode growth but probably due to direct exposure which caused lethal effects on juvenile and adult nematodes.

Malathion was extremely toxic to *in vitro* development of both 5B and L1C nematodes, although low numbers of bacteria were killed by $\geq$0.1 mg/ml, confluent bacterial growth was seen on 1.0 mg/ml plates. Since little direct toxicity to infective juveniles was observed and malathion severely inhibited *in vitro* growth, the protective effect of the nematode cuticle was evident. Exsheathed larvae and adults were sensitive to malathion at the tested concentrations. Both bacterial isolates were adversely affected by diazinon, as were infective juveniles. This dual effect combined to prevent *in vitro* growth at $\geq$0.001 mg/ml.

Carbaryl was not directly toxic to infective juveniles of 5B or L1C indicating the

protective effect of the cuticle. The bacterial isolates did not grow on $\geq$0.1 mg/ml carbaryl and it was at approximately this concentration that *in vitro* growth was arrested for both species. It was likely that in addition to the direct toxic effect on feeding nematodes, the depression of the bacterial population affected *in vitro* reproduction. Thus, this study has shown that some insecticides are directly lethal to the nematodes, while other compounds may indirectly cause nematode mortality by suppressing growth of symbiotically-associated bacteria.

In this study, nematodes exposed to low concentrations of insecticide were still able to infect and reproduce in *G. mellonella*, yet were unable to reproduce *in vitro*. This points out some of the inadequacies of the use of *in vitro* reproduction in assessing nematocide or insecticide toxicity to steinernematid nematodes. Removal of insecticide from exposed nematodes allows them to recover from any sublethal effects exerted on them by the insecticide and these nematodes remain infective (Hara and Kaya 1983a). In this study the nematodes retained infectivity if not lethally affected by insecticide. This occurred initially because removal from concentrated insecticide lowered immediate exposure. Additionally, after penetration into the insect, host metabolism may detoxify any remaining chemical.

4.5. MIGRATION IN INSECTICIDE-TREATED SOIL

Infective juvenile nematodes were attracted to insect larvae, as seen by significant migration of both L1C and 5B nematodes in soil towards *G. mellonella* larvae located 10 cm below their inoculation point. The kairomone or chemical responsible for eliciting favourable nematode response is unknown; carbon dioxide,

heat and fecal excrement have been implicated by other researchers (Schmidt and All 1979; Gaugler *et al.* 1980; Byers and Poinar 1982).

Nematode response to host stimuli appears to be rapid. Carbon dioxide moves rapidly through the air spaces in a soil matrix and movement of *S. feltiae* has been shown to be impaired in poorly-aerated, overly-saturated soil (Molyneaux and Bedding 1984). Soil columns used in this experiment were loosely packed and carbon dioxide contributed to nematode migration.

The proportion of L1C nematodes reaching the bottom level was lower than that of 5B, but the recovery of L1C was higher than 5B. These differences were due to strain or species differences. Soil moisture, texture and motility of the nematode have been shown to be important factors in nematode movement in soil (Schroeder and Beavers 1987).

The organochlorine insecticide, chlordane, and the organophosphate insecticide, diazinon, were consistently the most toxic compounds of the seven insecticides investigated in the previous experiments. Both were toxic to infective juveniles after immersion in aqueous suspension. In addition, *in vitro* development was impaired, as was bacterial growth over several of the tested concentrations.

Chlordane did not significantly impair migration of L1C or 5B in soil treated with $\leq$1.0 mg/ml. Diazinon significantly impaired migration of both L1C and 5B at 0.1 and 1.0 mg/ml. There was no direct toxicity at 0.1 mg/ml diazinon as noted previously. Therefore it was possible that at this non-lethal concentration, chemosensory response was impaired, a suggestion made by Marban-Mendoza and

Viglierchio (1980b), to explain insecticide-induced impairment of host attraction in the plant-parasitic nematode, *Pratylenchus vulnus*. They found that this nematode was attracted to bean roots but after exposure to the organophosphate, phenamiphos, the attraction was impaired at concentrations below those which inhibit motility.

It is likely that the physicochemical interactions of insecticides with soil components affected the bioavailability of the insecticides to the migrating nematodes. Nematodes migrate in the water film of the soil pores and thus, the water is the site of insecticide-nematode contact. Chlordane is highly hydrophobic and as such, tightly adsorbs to soil components, whereas diazinon is water soluble and thus, remains in the water fraction.

Diazinon is an anticholinesterase and can impair nematode motility, dispersion and attraction to host stimuli by disrupting neuromuscular function. Studies with plant-parasitic nematodes have implicated the somatic musculature as target sites of organophosphate insecticides (Croll 1974; Marbon-Mendoza and Viglierchio 1980a; Greco and Thomason 1980). The observations of this study do not provide direct evidence of the biochemical mode of action but the observed behavior of insecticide-treated nematodes (paralysis and decreased motility) are consistent with the proposed mechanism of insecticide action.

It was noted that although migration of L1C and 5B was not impaired by chlordane, nematode recovery from soil was significantly lower at 1.0 mg/ml for L1C and $\geq$0.1 mg/ml for 5B. This was due to a toxic effect exerted on the

nematodes when water was added to the soil to regain nematodes in the recovery process.

All insecticides were not compatible with the nematode/bacterial complex during *in vitro* growth experiments. In solution, the infective juveniles were directly exposed to insecticide and both organochlorines, as well as, diazinon were toxic at 1.0 mg/ml. Migration in soil was shown to be impaired by 0.1 and 1.0 mg/ml diazinon. Lower concentrations of chlordane, methoxychlor and diazinon as well as all tested levels of carbaryl, Matacil, Dimilin and malathion, did not impair infectivity and nematode reproduction in insect hosts. Clearly, assaying insecticides by the *in vitro* rearing methods employed in this study is not a reliable indicator of the potential success of these nematodes under field conditions.

The commercially-formulated chlordane used in this study is recommended for use against soil and lawn insects. The rate of application, as recommended by the manufacturer, varies from 2-8 mg/ml, depending on insect and spray area. Both L1C and 5B nematodes were sensitive to direct immersion in 1.0 mg/ml chlordane. Methoxychlor was also directly toxic to both juvenile nematodes at 1.0 mg/ml. This chemical is often used as an insecticide on fruit trees; dosage ranges from 2.2-3.8 mg/ml. The organophosphate, malathion, is used to control pests of vegetables and trees. Its recommended dosage is 0.5-1.5 mg/ml. In the current study, malathion was not directly toxic to either nematode at the tested concentrations. Diazinon is applied in dosages from 0.625-1.0 mg/ml to control fruit and garden insect pests and was found to be directly toxic to L1C and 5B

nematodes at 1.0 mg/ml. Carbaryl exposure resulted in low mortality at 1.0 mg/ml on 5B juveniles. It is applied on ornamental plants and domestic vegetable gardens for insect pest control. Depending on application area, the recommended dosage of carbaryl is 0.75-1.5 mg/ml. Both Matacil and Dimilin are used in foliar sprays to control insect pests in timber producing areas and on fruit trees. Traditionally, these chemicals are applied in spray programs in terms of an amount of chemical per given area. Both insecticides were not directly toxic to either L1C or 5B juveniles at 1.0 mg/ml.

Therefore, combined use of nematodes with the organochlorine insecticides methoxychlor and chlordane is not recommended, as spray programs use both chemicals at concentrations above juvenile nematode tolerance. Nematodes were not compatible with diazinon at the upper levels of recommended usage but the other organophosphate, malathion, was compatible at levels often used in the field. Carbaryl was compatible with both juvenile nematodes at field application levels.

Proposed use of entomogenous nematodes in integrated pest management programs shows much promise. High non-target toxicity and lengthy persistence in the environment have made the use of insecticides unfavourable. Recent advances in mass production techniques have made the use of steinernematid nematodes an attractive alternative. However, because of their sensitivity to lack of moisture, present use is limited to soil dwelling insect pests or those inhabiting cryptic habitats (Gaugler 1981). Because water evaporation is slower in soil relative to the surface of leaves and tree branches, it is an excellent medium for the moisture-sensitive steinernematid nematode (Simons and Poinar 1973).

The compatibility of insect growth regulators, organophosphates, and carbamate insecticides with other species of steinernematid nematodes and their associated entomophilic bacteria has been demonstrated (Pristavko 1967; Fedorko *et al.* 1977a, 1977b; Hara and Kaya 1983a). These insecticides have low environmental persistence and are preferable to the extremely persistent organochlorines (Kahn 1980). The strains of *Steinernema* sp. isolated from Newfoundland interact negatively with insecticides other than organochlorines (eg. diazinon). In presenting a point of contrast with the findings of others, this underscores the need to undertake compatibility tests in each ecological situation under evaluation for integrated pest management.

Insects have a higher sensitivity than nematodes to anticholinesterase insecticides and this factor is important in integrated programs (Pree *et al.* 1987). Nematodes are attracted to, infect and reproduce in moribund insect larvae which have been killed by insecticide (Hara and Kaya 1983b). The organophosphate and carbamate insecticides usually degrade within two weeks and this time factor can be important in integrated pest management. If L1C and 5B nematodes are to be used in integrated programs along with insecticides, compatibility must be achieved by altering the use pattern of the insecticides. Insecticide application rate must be lowered to have minimal effect on the nematode, but still affect the insect. Notwithstanding, nematodes can be applied to soil after a sufficient time has elapsed for insecticide degradation. The combination of insecticide and entomogenous nematodes should result in additive mortality of the pest species and result in the application of lowered amounts of insecticide required for insect control.

Summary and Conclusions

The indiscriminate use of insecticides during the past few decades has had adverse effects on the environment. While it cannot be ignored that several insecticides have contributed to the production of adequate quantities of food and the preservation of adequate standards of health, side-effects of insecticides on non-target organisms and on environmental quality have garnered concern for reduction in their use. By using both chemical and biocontrol strategies, the insect pest population can be effectively reduced with minimum damage to the environment.

The present study evaluated the compatibility of selected insecticides with two local steinernematid nematodes which have biocontrol potential. Information gathered may be useful for future field investigations. The conclusions of this study were;

- The double cuticle of the infective stage of the nematode acted as a barrier to almost all concentrations of the tested insecticides. However, this cuticle is a semi-permeable structure and could be penetrated. A toxic action was exerted on both nematode strains by both tested organochlorines and the organophosphate, diazinon.

- Except where insecticides had a lethal affect, no alteration in the efficacy of insecticide-treated juveniles was observed.

- The *in vitro* development of nematodes was impaired at several concentrations of all tested insecticides. Toxicity to both the exsheathed nematodes and the mutualistically-associated bacterium was responsible for reproductive failure.

- The number of 5B and L1C nematodes migrating in soil columns towards insect hosts was decreased in diazinon-treated soil. Chlordane did not significantly reduce migration of either L1C or 5B juvenile nematodes in soil, despite mortality after exposure to the highest tested concentration.

- The organochlorine insecticides, chlordane and methoxychlor and the organophosphate insecticide diazinon, caused mortality of both L1C and 5B juvenile nematodes at dosages recommended for field usage. Application levels of these insecticides must be reduced when nematodes are to be used in conjunction with the chemicals.

- The organophosphate insecticide, malathion, was compatible with both nematodes at dosages recommended for field use and thus is a suitable chemical for integrated pest management programs involving the investigated steinernematids. The carbamate insecticides, carbaryl and Matacil as well as the insect growth regulator, Dimilin, were compatible with the juvenile steinernematid nematodes examined in this study.

The steinernematid nematodes examined in this study were generally compatible with the insecticides tested. This allows future researchers an information base that can be used if an integrated program of insecticides and nematodes is implemented for more effective pest management. In cases where compatibility was not shown, it may be possible to delay application of the nematodes to allow time for degradation of insecticide. Clearly, the concomitant use of steinernematid nematodes and selected insecticides shows promise.

REFERENCES

Akhurst, R.J. 1980. Morphological and Functional Dimorphism in *Xenorhabdus* spp., Bacteria Symbiotically Associated with the Insect Pathogenic Nematodes *Neoaplectana* and *Heterorhabditis*. J. Gen. Micro. 121:303-309.

Akhurst, R.J. 1982. Antimicrobial Activity of *Xenorhabdus* spp., Bacteria Symbiotically Associated with Insect Pathogenic Nematodes of the Families Heterorhabditidae and Steinernematidae. J. Gen. Micro. 128:3061-3066.

Akhurst, R.J. 1983. *Neoaplectana* species: Specificity of Association with Bacteria of the Genus *Xenorhabdus*. Exp. Parasitol. 55:258-263.

Akhurst, R.J. 1986. *Xenorhabdus nematophilus* subsp. *poinarii*: Its Interaction with Insect Pathogenic Nematodes. System. Appl. Microbiol. 8:142-147.

Akhurst, R.J. and N.E. Boemare. 1986. A Non-luminescent Strain of *Xenorhabdus luminescens* (Enterobacteriaceae). J. Gen. Micro. 132:1917-1922.

Alikhan, M.A., A. Bednarek and S. Grabiec. 1985. The Physiological and Morphological Characteristics of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) in Two Insect Hosts. J. Invertebr. Pathol. 45:168-173.

Barka, T. and P.J. Anderson. 1965. Histochemistry: Theory, Practice and Bibliography. Hoeber Medical Division, Harper and Row Publ. Inc., N.Y.

Bedding, R.A. 1976. New Methods Increase the Feasibility of Using *Neoaplectana* spp. (Nematoda) for the Control of Insect Pests. Proc. 1st Int. Coll. Invertebr. Path. Kingston, Canada: 250-254

Bedding, R.A. 1981. Low Cost, *In Vitro* Mass Production of *Neoaplectana* and *Heterorhabditis* Species, (Nematoda) for Field Control of Insect Pests. Nematologica 27:109-114.

Bedding, R.A. 1984. Large Scale Production, Storage and Transport of the Insect-Parasitic Nematodes *Neoaplectana* spp. and *Heterorhabditis* spp. Ann. Appl. Biol. 104:117-120.

Burman, M. and A.E. Pye. 1980. *Neoaplectana carpocapsae*: Movements of Nematode Populations On a Thermal Gradient. Exp. Parasitol. 49:258-265.

Burman, M., A.E. Pye and N.O. Nojd. 1979. Preliminary Field Trail of the Nematode *Neoaplectana carpocapsae* Against Larvae of the Large Pine Weevil, *Hylobius abietus* (Coleoptera:Curculionidae). Ann. Entomol. Fennici 45:88.

Byers, J.A. and G.O. Poinar Jr. 1982. Location of Insect Host by the Nematode, *Neoaplectana carpocapsae*, in Response to Temperature. Behaviour 79:1-10.

Calcott, P. and R. Fatig. 1984. Inhibition of Chitin Metabolism by Avermectin in Susceptible Organisms. J. Antibiot. 37:253-259.

Cheng, H.H. and G.F. Bucher. 1972. Field Comparison of the Neoaplectanid Nematode DD-136 with Diazinon for Control of *Hylemya* spp. on Tobacco. J. Econ. Entomol. 65:1761-1763.

Croll, N.A. 1974. Indolealkylamines in the Coordination of Nematode Behaviour Activities. Can. J. Zool. 53:894-903.

Croll, N.A. 1977. Sensory Mechanisms in Nematodes. Ann. Rev. Phytopathol. 15:75-89.

Dutky, S.R. 1956. Nematode on Our Side. Agric. Res. 40:3-4.

Dutky, S.R. 1959. Insect Microbiology. Adv. Appl. Microbiol. 1:175-200.

Dutky, S.R., J.V. Thompson and G.E. Cantwell. 1962. A Technique for Mass Rearing the Greater Wax Moth. Proc. Ent. Soc. Wash. 64:56-58.

Dutky, S.R., J.V. Thompson and G.E. Cantwell. 1964. A Technique for Mass Propagation of the DD-136 Nematode. J. Insect. Pathol. 6:417-422.

Fedorko, A., M. Kamionek, J. Kozlowska and E. Mianowska. 1977a. The Effects of Some Insecticides on Nematodes of Different Ecological Groups. Pol. Ecol. Stud. 3:79-88.

Fedorko, A., M. Kamionek, J. Kozlowska and E. Mianowska. 1977b. The Effect of Vydate-oxamyl on Nematodes of Different Ecological Groups. Pol. Ecol. Stud. 3:89-93.

Finney, J.R. 1984. Alternative Sources of Steinernematid Nematodes for use as Biocontrol Agents Against Insect Pests in Newfoundland. CANUSA Newsletter 34:5.

Gaugler, R. 1981. Biological Control Potential of Neoaplectanid Nematodes. J. Nematol. 13:241-249.

Gaugler, R., L. LeBeck, B. Nakagaki and G.M. Boush. 1980. Orientation of the Entomogenous Nematode *Neoaplectana carpocapsae* to Carbon Dioxide. Environ. Entomol. 9:649-652.

Georgis, R. and G.O. Poinar Jr. 1983a. Effect of Soil Texture on the Distribution and Infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). J. Nematol. 15:308-311.

Georgis, R. and G.O. Poinar Jr. 1983b. Effect of Soil Texture on the Distribution and Infectivity of *Neoaplectana glaseri* (Nematoda: Steinernematidae). J. Nematol. 15:329-332.

Georgis, R. and G.O. Poinar Jr. 1983c. Vertical Migration of *Heterorhabditis bacteriophora* and *H. heliothidis* (Nematoda:Heterorhabditidae) in Sandy Loam Soil. J. Nematol. 15:652-654.

Glaser, R.W. 1931. The Cultivation of a Nematode Parasite of an Insect. Science 73:614-615.

Goodell, P.B. 1982. Soil Sampling and Processing for Detection and Quantification of Nematode Populations for Ecological Studies. Pp. 178-198. *In* Nematodes in Soil Ecosystems. Ed. D.W. Freckman. Univ. Texas Press, Austin.

Greco, N. and I.J. Thomason. 1980. Effect of Phenamiphos on *Heterodera schactii* and *Meloidogyne javanica*. J. Nematol. 12:91-96.

Hamilton, W.A. 1971. Membrane Active Antibacterial Compounds. Pp. 77-93. *In* Inhibition and Destruction of the Microbial Cell. Ed. W.B. Hugo. Academic Press, N.Y.

Hansen, E.L. and J.W. Hansen. 1978. Nematode Parasites in Animals and Plants. *In* Methods of Cultivating Parasites *In Vitro*. Eds. A.R. Taylor and J.R. Baker. Academic Press, London.

Hara, A.H. and H.K. Kaya. 1982. Effects of Selected Insecticides on the *In Vitro* Development of the Entomogenous Nematode *Neoaplectana carpocapsae*. J. Nematol. 14:486-491.

Hara, A.H. and H.K. Kaya. 1983a. Toxicity of Selected Organophosphate and Carbamate Pesticides to Infective Juveniles of the Entomogenous Nematode *Neoaplectana carpocapsae* (Rhabditida:Steinernematidae). Env. Entomol. 12:496-510.

Hara, A.H. and H.K. Kaya. 1983b. Development of the Entomogenous Nematode, *Neoaplectana carpocapsae* (Rhabditida:Steinernematidae), in Insecticide-killed Beet Armyworm(Lepidoptera:Noctuidae). J. Econ. Entomol. 76:423-426.

Hara, A.H., J.E. Lindegren and H.K. Kaya. 1981. Monoxenic Mass Production of the Entomogenous Nematode *Neoaplectana carpocapsae* Weiser on Dogfood/Agar Medium. USDA/SEA. Adv. Agric. Technol. Western Ser., AAT-W-16.

Harlan, D.P., S.R. Dutky, G.R. Padgett, J.A. Mitchell, Z.A. Shaw and E.J. Bartlett. 1971. Parasitism of *Neoaplectana dutkyi* in White-fringed Beetle Larvae. J. Nematol. 3:280-283.

Hooper, D.J. 1986. Extraction of Free-living Stages from Soil. *In* Laboratory Methods for Work with Plant and Soil Nematodes. Ed. J.F. Southey. Ministry of Agriculture, Fisheries and Food. Her Majesty's Stationery Office, London.

House, H.L., H.E. Welch, and T.R. Cleugh. 1965. Food Medium of Prepared Dog Biscuit for the Mass-production of the Nematode DD-136(Nematoda: Steinernematidae). Nature 206:847.

Huettel, R.N. 1986. Chemical Communicators in Nematodes. J. Nematol. 18:3-8.

Ishibashi, N. and E. Kondo. 1986. *Steinernema feltiae* (DD-136) and *S. glaseri*:

Persistence in Soil and Bark Compost and Their Influence on Native Nematodes. J. Nematol. 18:310-316.

Kahn, S.U. 1980. Pesticides in the Soil Environment. Elsevier Scientific Pub., Co., Amsterdam.

Kamionek, M. 1979. Influence of Pesticides on the Mortality and Affectiveness of *Neoaplectana carpocapsae* Weiser. Pp. 87-88 *In* Proceedings of the International Colloquium on Invertebrate Pathology and 11th Annual Meeting Society for Invertebrate Pathology. Prague, Czech.

Kaya, H.K. 1977. Development of DD-136 strain of *Neoaplectana carpocapsae* at constant temperatures. J. Nematol. 9:346-349.

Keetch, D.P. 1974. The Effect of Nematicides on Feeding, Posture and Dispersal of *Aphelenchus avenae*. Nematologica 20:107-118.

Lawrence, J.V. and S. Maier. 1977. Correction for the Inherent Error in Optical Density Readings. Appl. Env. Micro. 33:482-484.

Lewis, L.C. and E.S. Raun. 1978. Laboratory and Field Evaluation of the DD-136 Strain of *Neoaplectana carpocapsae* for Control of the European Corn Borer, *Ostrina nubilalis*. Iowa State J. Res. 52:391-396.

Lindegren, J.E., D.F. Hoffman, S.S. Collier and R.D. Fries. 1979. Propagation and Storage of *Neoaplectana carpocapsae* Weiser Using *Amyelois transitella* (Walker) Adults. USDA\SEA. Adv.Argric. Tech. Western Series No. 3.

Lysenko, O. and J. Weiser. 1974. Bacteria Associated with the Nematode *Neoaplectana carpocapsae* and the Pathogenicity of this Complex for *Galleria mellonella* Larvae. J. Invertebr. Pathol. 24:332-336.

Marban-Mendoza, N. and D.R. Viglierchio. 1980a. Behaviour Effects of Carbofuran and Phenamiphos on *Pratylenchus vulnus* I. Motility and Dispersion J. Nematol. 12:102-114.

Marban-Mendoza, N. and D.R. Viglierchio. 1980b. Behaviour Effects of Carbofuran and Phenamiphos on *Pratylenchus vulnus* II. Attraction to Bean Roots. J. Nematol. 12:114-118.

Marban-Mendoza, N. and D.R. Viglierchio. 1980c. Behaviour Effects of Carbofuran and Phenamiphos on *Pratylenchus vulnus* III. Penetration and Development. J. Nematol. 12:119-129

Molyneaux, A.S. and R.A. Bedding. 1984. Influence of Soil Texture and Moisture on the Infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for Larvae of the Sheep Blowfly, *Lucilia cuprina*. Nematologica 30:358-365.

Peacock, C.H. and R.A. Dunn. 1986. Effects of Nematicide Formulations on Turfgrass Nematodes. Soil and Crop Sci. Soc. Florida Proc. 45:185-189.

Pelczar, H.G. and R.D. Reid. 1972. Microbiology. McGraw-Hill Book Co., N.Y.

Poinar, G.O. Jr. 1966. The Presence of *Achromobacter nematophilus* in the Infective Stage of a *Neoaplectana* sp. (Steinernematidae:Nematoda) Nematologica 12:105-108.

Poinar, G.O. Jr. 1979. Nematodes for Biological Control of Insects. CRC Press Boca Raton, Florida.

Poinar, G.O. Jr. 1983. The Natural History of Nematodes. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Poinar, G.O. Jr. 1986. Entomogenous Nematodes. Pp. 95-121. Fortschritte der Zoologie Bd. 32 Franz (Hrsg.): Biological Plant and Health Protection Ed. G. Fisher Verlag Stuttgart, N.Y.

Poinar, G.O. Jr. and G.M. Thomas. 1966. Significance of *Achromobacter nematophilus* Poinar and Thomas (Achromobacteraceae:Eubacteriales) in the Development of the Nematode, DD-136 (*Neoaplectana* sp. Steinernematidae). Parasitol. 56:385-390.

Poinar, G.O. Jr. and G.M. Thomas. 1967. The Nature of *Achromobacter nematophilus* as an Insect Pathogen. J. Invertebr. Pathol. 9:510-514.

Prakasa Rao, P.S., P.K. Das and G. Padhi. 1975. Note on Compatibility of DD-136 (*Neoaplectana dutkyi*), an Insect Parasitic Nematode with Some Insecticides and Fertilizers. Indian J. Agric. Sci. 45:275-277.

Pree, D.J., J.L. Townshend and D.E. Archibald. 1987. Sensitivity of Acetylcholinesterases from *Aphelenchus avenae* to Organophosphorus and Carbamate Pesticides. J. Nematol. 19:188-193.

Pristavko, V.P. 1967. On the Use of Entomopathogenic Bacteria Together With Insecticides in the Control of Insect Pests. Entomol. Rev. 46:443-446.

Pye, A.E. and M. Burman. 1981. *Neoaplectana carpocapsae*: Nematode Accumulations on Chemical and Bacterial Gradients. Exp. Parasitol. 51:13-20.

Schlegel, H.G. 1986. General Microbiology. Cambridge Univ. Press, N.Y.

Schmidt, J. and J.N. All. 1978. Chemical Attraction of *Neoaplectana carpocapsae* (Nematoda:Steinernematidae) to Insect Larvae. Environ. Entomol. 7:605-607.

Schmidt, J. and J.N. All. 1979. Attraction of *Neoaplectana carpocapsae* (Nematoda:Steinernematidae) to Common Excretory Products of Insects. Environ. Entomol. 8:55-61.

Schmiege, D.C. 1963. The Feasibility of Using a Neoaplectanid Nematode for Control of Some Forest Insect Pests. J. Econ. Entomol. 56:427-431.

Schroeder, W.J. and J.B. Beavers. 1987. Movement of the Entomogenous Nematodes of the Families Heterorhabditidae and Steinernematidae in Soil. J. Nematol. 15:173-182.

Shepard, M. (Ed.). 1973. Pest Management Systems. Subcommitee on Insect Pests, Academy of Sciences. *In* Insect Pest Management. Pp. 269., MSS Inf. Corp.

Simons, W.R. and G.O. Poinar Jr. 1973. The Ability of *Neoaplectana carpocapsae* (Steinernematidae:Nematoda) to Survive Extended Periods of Desiccation. J. Invertebr. Pathol. 22:228-230.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Co., San Francisco.

Steele, A.E. and L.R. Hodges. 1975. *In Vitro* and *In Vivo* Effects of Aldicarb on Survival and Development of *Heterodera schachtii*. J.Nematol. 7:303-312.

Thomas, G.M. and G.O. Poinar Jr. 1979. *Xenorhabdus* gen. nov., a Genus of Entomopathogenic Nematophillic Bacteria of the Familiy Enterobacteriaceae. Int. J. System. Bact. 29:352-360.

Triggiani, O. and G.O. Poinar Jr. 1976. Infection of Adult Lepidoptera by *Neoaplectana carpocapsae* (Nematoda). J. Invertebr. Pathol. 27:413-414.

Veech, J.A. 1978a. The Effects of Diflubenzuron on Egg Formation by the Root-Knot Nematode. J. Nematol. 10:208-209.

Veech, J.A. 1978b. The Effects of Diflubenzuron on the Reproduction of Free Living Nematodes. Nematologica 24:312-320.

Wallace, H.R. 1958a. Movement of Eelworms I. The Influence of Pore Size and Moisture Content of the Soil on the Migration of Larvae of the Beet-Eelworm, *Heterodera schachtii* Schmidt. Ann. Appl. Biol. 46:74-85.

Wallace, H.R. 1958b. Movement of Eelworms III. The Relationship Between Eelworm Length, Activity and Mobility. Ann. Appl. Biol. 46:662-668.

Wallace, H.R. 1960. Movement of Eelworms VI. The Influences of Soil Type, Moisture Gradients and Host Plant Roots on the Migration of the Potato- Root Eelworm *Heterodera rostochiensis* Wollenweber. Ann. Appl. Biol. 48:107-120.

Wouts, W.M. 1981. Mass Production of the Entomogenous Nematode *Heterorhabditis heliothidis* (Nematoda:Heterorhabditdae) on Artificial Media. J. Nematol. 13:467-469.

Zar, J.H. 1984. Biostatistical Analysis. Prentice-Hall Inc., Englewood Cliffs, N.J.