

EFFECT OF ENTOMOPATHOGENIC NEMATODES ON
THE CABBAGE MAGGOT, DELIA RADICUM L., AND THEIR
BEHAVIOR IN THE PRESENCE OF IT AND ITS HOST PLANTS

by

Zucheng Lei

B. Sc. Agr., South China Agricultural University, 1985.

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in the Department
of
Biological Sciences

© ZUCHENG LEI 1990

SIMON FRASER UNIVERSITY

April, 1990

All rights reserved. This work may not be
reproduced in whole or in part, by photocopy
or other means, without the permission of the author

APPROVAL

Name: ZUCHENG LEI

Degree: Master of Science

Title of Thesis:

EFFECT OF ENTOMOPATHOGENIC NEMATODES ON THE CABBAGE MAGGOT, DELIA RADICUM L.,
AND THEIR BEHAVIOR IN THE PRESENCE OF IT AND ITS HOST PLANTS

Examining Committee:

Chairman: Dr. C.L. Kemp, Associate Professor, Dept. of Biological Sciences

Dr. J.M. Webster, Professor, Senior Supervisor,
Dept. of Biological Sciences, SFU

Dr. H.R. MacCarthy, Adjunct Professor, Dept. of
Biological Sciences, SFU

Dr. T.A. Rutherford, Research Associate, Dept. of
Biological Sciences, SFU

Dr. H. Kaya, Dept. of Nematology, University of
California, Public Examiner

Date Approved

18 April, 1990

PARTIAL COPYRIGHT LICENSE

I hereby grant to Simon Fraser University the right to lend my thesis, project or extended essay (the title of which is shown below) to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users. I further agree that permission for multiple copying of this work for scholarly purposes may be granted by me or the Dean of Graduate Studies. It is understood that copying or publication of this work for financial gain shall not be allowed without my written permission.

Title of Thesis/Project/Extended Essay

EFFECT OF ENTOMOPATHOGENIC NEMATODES ON THE CABBAGE MAGGOT,

DELIA RADICUM L., AND THEIR BEHAVIOR IN THE PRESENCE OF IT

AND ITS HOST PLANTS

Author:

(signature)

ZUCHENG LEI

(name)

April 19, 1990

(date)

ABSTRACT

Two species/strains of Steinernema and two of Heterorhabditis were studied for use as biological control agents against the cabbage maggot, Delia radicum. The NC162 strain of Heterorhabditis sp. was the most virulent against 1st- and 2nd-instar D. radicum. The egg shells and puparia of D. radicum were impenetrable to these nematodes. The newly-hatched maggots did not appear to be infected by the nematodes before they entered a host plant; the maggots entered the upper root of radish in less than 6 h, during which time only 11 out of 200 maggots were killed; and within 24 and 48 h, only 18% and 71% respectively of the maggots were killed by NC162 in Petri dishes. NC162 was more effective than T327 against 3rd-instar maggots. Carbon dioxide treatment of 3rd-instar maggots for 8 h pacified the maggots so that NC162 killed them within 12 h at doses of 5,000 and 500 nematodes per dish. Adult flies of D. radicum, especially untanned ones, were susceptible to NC162. In the field, NC162 reduced the population of D. radicum, and its damage in radishes. A similar experiment on kohlrabi was inconclusive.

The behavior of T327 and NC162 in the presence of D. radicum and its host plants was investigated on thin Bacto Agar in Petri dishes. Newly-formed puparia and frozen dead maggots were attractive to both strains. Two-month-old T327 reached the insect targets within 15 min. But newly-harvested T327 did not respond to the insect targets whereas those of NC162 did. When both strains had been stored for 2 months, T327 responded faster and more reached the targets of four larval D. radicum than did

NC162. There were no significant differences between larval D. radicum and Galleria mellonella (the greater wax moth) in their ability to attract T327. Generally, there was a tendency for a greater number of insects to attract more nematodes. Germinated seeds of radishes and the roots of ball cabbage and radishes, were attractive to T327, but rutabaga roots neither attracted nor repelled the nematodes. The possible factors responsible for attracting the nematodes to these insects and plants are discussed.

**To my parents
and my motherland,
the People's Republic of China.**

ACKNOWLEDGEMENTS

I thank Dr. J.M. Webster for supporting my research and for his encouragement, tireless guidance throughout the preparation of this thesis. I express my gratitude to my motherland (The People's Republic of China) for providing me the opportunity and supporting my studies in this beautiful country--Canada. Many thanks are due to the other members of my Supervisory Committee, Drs. H.R. MacCarthy and T.A. Rutherford, for their numerous helpful suggestions for my work and critical corrections of the manuscripts. I wish to thank Dr. S. Zervos for many helpful suggestions, Mr. Akbar Syed for providing Delia radicum and for his expert advice in rearing this insect, Ms. Monica Locher and Ms. Donna Bartlett for maintaining a source of nematodes, Mr. Mike Wagner and Mr. Edmund Canaria for their assistance in harvesting the field experiment, Dr. Vic Bourne for his advice in making photographs, Mr. M.E. Sweeney (Horticulturist, Ministry of Agriculture and Food, Abbotsford, B.C.) for the latest information on this insect pest in B.C., and especially my wife (Lena Zhang) for her encouragement and for typing some of the references. Finally, I acknowledge Simon Fraser University and the Department of Biological Sciences for providing the facilities to complete my studies, and the Natural Sciences and Engineering Research Council for additional funding of the research.

TABLE OF CONTENTS

Approval	ii
Abstract	iii
Dedication	v
Acknowledgements	vi
Table of Contents	vii
List of Tables	x
List of Figures	xi
List of Appendixes	xvi
Chapter 1: Introduction	1
Chapter 2: General Materials and Methods	20
1. The Cabbage Maggot, <u>D. radicum</u>	20
a. Source	20
b. Rearing <u>D. radicum</u>	20
2. The Nematodes	24
a. Source and rearing	24
b. Counting nematodes	25
3. Host Plants of <u>D. radicum</u>	26
Chapter 3: Experiments and Results	27
A. Selection of Nematode Strains for Controlling the Cabbage Maggot	27
B. Behavior of the Nematodes in the Presence of Larval and Pupal <u>D. radicum</u>	30
1. Qualitative experiments	30
(1). Experiment with puparia	30
(2). Experiment with larvae	31
2. Quantitative experiments	35
(1). Experiment with puparia	35

a. Using two-month-old infective nematodes	35
b. Using newly-harvested and one-month-old infective nematodes	39
(2). Experiment with larvae	40
C. Using Selected Nematode Strains to Control the Cabbage Maggot in the Laboratory	48
1. Eggs	48
2. First-Instar Larvae	51
(1). Mortality test of the first-instar larvae of <u>D. radicum</u>	51
(2). Time for newly-hatched maggots to enter a host plant	53
(3). How effective are the nematodes on newly- hatched maggots within 6 hours?	53
3. Third-Instar Larvae	55
(1). Comparison of efficacy between strains NC162 and T327	55
(2). Using CO ₂ -pretreated and normal larvae	56
4. Puparia	63
(1). Using 1-day-old puparia	63
(2). Using mature, 3-instar larvae	64
5. Adults	65
D. Behavior of Nematodes in the Presence of Host Plants Used by <u>D. radicum</u>	68
1. In the presence of germinated seeds	70
2. In the presence of roots	73
E. Field Trial	74

Chapter 4: Discussion	83
A. Selection of Nematode Strains for Controlling the Cabbage Maggot	83
B. Behavior of the Nematodes in the Presence of <u>D. radicum</u> and Its Host Plants	86
1. In the presence of <u>D. radicum</u>	86
2. In the presence of host plants used by <u>D. radicum</u>	91
C. Using Selected Nematode Strains to Control the Cabbage Maggot in the Laboratory	93
D. Field Trial	96
Chapter 5. Conclusions	102
References Cited	104
1. On the Cabbage Maggot	104
2. On the Nematodes	108
3. On Statistics	115
Appendixes	116

LIST OF TABLES

- Table 1. P values from the comparisons of percentages of introduced, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of one or four larval Delia radicum (DR) or Galleria mellonella (GM), and targets of the control with no insects, within 24 h, at 20-23.5 C. 50
- Table 2. P values* from the comparisons of cumulative mortality of 3rd-instar larval Delia radicum (including subsequently-formed puparia), pretreated with CO₂ (CO₂) and normal (N), then exposed to two doses (5,000 and 500) of infective juvenile Heterorhabditis strain NC162 for 4 days, at 21.3-22.3 C. 62
- Table 3. Numbers of infective juvenile Heterorhabditis strain NC162 found in the heads, thoraxes, and abdomens of 57 adult Delia radicum, after 6 days of exposure, at 19-27 C. 69

LIST OF FIGURES

- Fig. 1. Maintaining a colony of Delia radicum at 20-25 C, with a photoperiod of 16 h a day. A: adult food; B: water; C: an oviposition site. 21
- Fig. 2. A culture for rearing larval and pupal Delia radicum at 20-25 C: (a) newly-laid eggs have been introduced into the moist sand and (b) after about 2 weeks, mature larvae coming out from a rutabaga and pupating in the sand. 23
- Fig. 3. Day/mean cumulative mortality curves for Delia radicum, exposed to four nematode strains, namely DD136 and Breton of Steinernema, and NC162 and A13-5 of Heterorhabditis, for 6 days, at 16.5-24.5 C. Top bars stand for standard errors (SE) (n=5). 29
- Fig. 4. Tracks showing the orientation of infective juvenile Heterorhabditis heliothidis strain T327 (at three per dish) towards frozen, dead, third-instar larval Delia radicum (at two per dish), over time, at 21C, on thin agar: (a) after 5 min and (b) after 15 min; 32
 (c) after 30 min and (d) after 1 h; 33
 (e) after 1.5 h and (f) after 4 h. 34
- Fig. 5. Mean percentages of introduced, infective juvenile Heterorhabditis heliothidis strain T327 (at three per dish), which reached newly-formed puparia of Delia radicum, over time, at 21 C. Top bars stand for standard errors (SE) (n=13). .. 36
- Fig. 6. Mean percentages of introduced, infective juvenile Heterorhabditis heliothidis strain T327 (at three per dish),

which reached frozen, dead, third-instar larval Delia radicum, over time, at 21 C. The bars stand for standard errors (SE) (n=12). 37

Fig. 7. Arrangement of infective juvenile Heterorhabditis strain T327 or NC162, and of pupal or larval Delia radicum on filter paper discs, on agar in a Petri dish. N= nematode introduction point; 1, 2, & 4= numbers of insects; C= control. 38

Fig. 8. Arrangement of larval Delia radicum (DR) and Galleria mellonella (GM) on filter paper discs, and of infective juvenile Heterorhabditis heliothidis strain T327, on agar in a Petri dish. N= nematode introduction point; 1 & 4= numbers of insects; C=control. 41

Fig. 9. Mean percentages of introduced, two-month-old, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of one (1 P), or two (2 P), or four (4 P) newly-formed puparia of Delia radicum, and targets of the control (C), within 4 h, at 24-24.5 C. Top bars stand for standard errors (SE) (n=10). 42

Fig. 10. Mean percentages of introduced, newly-harvested, infective juvenile Heterorhabditis strain NC162, which reached targets of one (1 P) or two (2 P), or four (4 P) newly-formed puparia of Delia radicum, and targets of the control (C), within 24 h, at 23.5-25.8 C. Top bars stand for standard errors (SE) (n=10). 44

Fig. 11. Mean percentages of introduced, one-month-old, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of one (1 P), or two (2 P), or four (4 P)

newly-formed puparia of Delia radicum, and targets of the control (C), after 4 h and 24 h, at 23.5-25 C and 23.3-25.1 C, respectively. Top bars stand for standard errors (SE) (n=10). 45

Fig. 12. Mean percentages (\pm SE) (n=10) of introduced, infective juveniles of strain T327 or NC162 of Heterorhabditis, which reached targets of one (1 L), or two (2 L), or four (4 L) frozen, dead, 3rd-instar larval Delia radicum, and targets of the control (C), within 24 h. 47

Fig. 13. Mean percentages of introduced, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of one (1 L) or four (4 L) larval Delia radicum (DR) and Galleria mellonella (GM) (ten replicates for each species), and targets of the control (C) (twenty replicates), within 24 h, at 20-23.5 C. Top bars stand for standard errors (SE). 49

Fig. 14. Mean cumulative mortality of 1st-instar larval Delia radicum exposed to infective juvenile Heterorhabditis strain NC162, within 24 h and 48 h, at 20-22 C. Top bars stand for standard errors (SE) (n=10). 54

Fig. 15. Mean cumulative mortality of 3rd-instar larval Delia radicum exposed to infective juvenile Heterorhabditis strain NC162 or T327 for 7 days, at 20-24.8 C. Bars stand for standard errors (SE) (n=10). 58

Fig. 16. A 3rd-instar larval Delia radicum infected with Heterorhabditis strain NC162, containing the developing nematodes. 60

- Fig. 17. Mean cumulative mortality of 3rd-instar larval Delia radicum (including subsequently-formed puparia), pretreated with CO₂ (CO₂) and normal (N), then exposed to two doses (5,000 and 500) of infective juvenile Heterorhabditis strain NC162 for 4 days, at 21.3-22.3 C. Bars stand for standard errors (SE) (n=5). 61
- Fig. 18. Mean cumulative mortality of adult Delia radicum exposed to infective juvenile Heterorhabditis strain NC162 for 6 days, at 19-27 C. Bars stand for standard errors (SE) (n=15). 67
- Fig. 19. Arrangement of infective juvenile Heterorhabditis heliothidis strain T327, and (a) germinated seeds of radish; (b) cylinders of radish and rutabaga roots, or of radish and ball cabbage roots, on filter paper discs on agar in Petri dishes. N= nematode inoculation point; 1 & 3= number of seeds; RC= root cylinders; C= controls. 71
- Fig. 20. Mean percent (\pm SE) infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of germinated seeds of radish (ten replicates), and targets of the control (twenty replicates), within 3 h, at 24-25 C. 72
- Fig. 21. Mean percent infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of cylinders of: (a) radish and rutabaga roots (ten replicates), and targets of the control (twenty replicates), at 23.8-24.2 C; and (b) radish and ball cabbage roots (ten replicates), and targets of the control (twenty replicates), at 23-24.4 C; within 2.5 h. Top bars stand for standard errors (SE). 75

- Fig. 22. Percent larvae and puparia of Delia radicum recovered from the soil and roots of (a) radishes, and (b) kohlrabi, in the field treated with infective juvenile Heterorhabditis strain NC162 (at 5,000 per plant in plot I and 50,000 per plant in plot II), and from controls I and II. 80
- Fig. 23. Percent (a) radishes and (b) kohlrabi roots, with grade-0-5 damage caused by Delia radicum in the field treated with infective juvenile Heterorhabditis strain NC162, at 5,000 per plant in treatment I and 50,000 per plant in treatment II, and in controls I and II. (Explanation of the "Damage grades" is given in the text). 81

LIST OF APPENDIXES

- Appendix 1. Infectivity of four nematode strains, namely DD136 and Breton of Steinernema, and NC162 and A13-5 of Heterorhabditis for first- and second-instar larval Delia radicum. There were five replicates (n=5) for each strain.
- Appendix 2. The number of nematodes, at three per dish, of Heterorhabditis heliothidis strain T327 which were present at newly-formed puparia of Delia radicum over time, at 21 C.
- Appendix 3. The number of nematodes, at three per dish, of Heterorhabditis heliothidis strain T327 which were present at frozen, dead, 3rd-instar larvae of Delia radicum over time, at 21 C.
- Appendix 4. Percentages of introduced, two-month-old, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of newly-formed puparia of Delia radicum, and targets of the control, within 4 h, at 24-24.5 C
- Appendix 5. Percentages of introduced, newly-harvested, infective juvenile Heterorhabditis strain NC162, which reached targets of newly-formed puparia of Delia radicum, and targets of the control, within 24 h, at 23.5-25.8 C.
- Appendix 6. Percentages of introduced, one-month-old, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of newly-formed puparia of Delia radicum, and targets of the control, within (a) 4 h at 23.5-25 C, and (b) 24 h at 23.3-25.1 C.
- Appendix 7. Percentages of introduced, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of larval

Delia radicum and Galleria mellonella, and targets of the control, within 24 h, at 20-23.5 C.

Appendix 8. Numbers of dead maggots and numbers of maggots parasitized (in parentheses) by nematodes after exposing 1st-instar larval Delia radicum (at 10 per dish) to infective juvenile Heterorhabditis strain NC162, for 24 h and 48 h, at 20-22 C.

Appendix 9. The fate of 3rd-instar larval Delia radicum exposed to infective juvenile Heterorhabditis strain NC162 or T327, for 7 days, at 20-24.8 C.

Appendix 10. Cumulative numbers of dead Delia radicum, pretreated with CO₂ (CO₂) and normal (N), then exposed to infective juvenile Heterorhabditis strain NC162 (at 500 or 5,000 per dish), and in the control (no nematodes), for 4 days, at 21.3-22.3 C.
L= 3rd-instar larvae; P= subsequently-formed puparia.

Appendix 11. The fate of adult Delia radicum exposed to infective juvenile Heterorhabditis strain NC162, and in the control, for 6 days, at 19-27 C.

Appendix 12. Percentages of infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of germinated seeds of radish (cv Cherry Belle), and of the control, within 3 h, at 24-25 C.

Appendix 13. Percentages of infective juvenile Heterorhabditis heliothidis strain T327, which within 2.5 h reached: (a) root cylinders of radish and rutabaga, and the control, at 23.8-24.2 C; (b) root cylinders of radish and ball cabbage, and the control, at 23-24.4 C.

Appendix 14. The results from the field trial treated with infective juvenile Heterorhabditis strain NC162: (a) number of larvae and puparia of Delia radicum recovered from the soil and radishes; (b) wet weight and number of radishes damaged by Delia radicum. (Explanation of the "Damage grades" is given in the text).

Appendix 15. The results from the field trial treated with infective juvenile Heterorhabditis strain NC162: (a) number of larvae and puparia of Delia radicum recovered from the soil and roots of kohlrabi; (b) wet weight and number of kohlrabi damaged by Delia radicum. (Explanation of the "Damage grades" is given in the text).

Appendix 16. Correspondence from Dr. J. Curran (CSIRO).

CHAPTER 1: INTRODUCTION

Delia radicum Linnaeus is commonly called the cabbage maggot in North America (Muesebeck, 1942). It was described by Bouché in 1833 as Anthomyia brassicae. The specific name was generally accepted but the generic name was not, and according to Coaker and Finch (1971), there were five in regular use until recently: Hylemyia in the U.S.A. and Canada; Chortophila or Phorbia or Delia in France, Germany, and U.S.S.R.; Erioischia or Delia in the U.K. Currently, the name of this insect has reverted to the original of Linnaeus--Delia radicum, in which the name radicum is a clear reference to the larval habitat (Pont, 1981).

This insect is restricted to the temperate zone of the holarctic region (36°-60° N) (Schoene, 1916). It has been a destructive pest in Eastern Canada since about 1855. Not recorded from Saskatchewan until 1952 or from Manitoba until 1958, it now occurs in vegetable-growing areas throughout Canada, including the North West Territories (Beirne, 1971).

It is the larval D. radicum that cause damage by feeding and tunneling, mostly in the main roots but sometimes in the aerial parts of many cruciferous species, whether cultivated or wild (Coaker and Finch, 1971). The following crops, approximately in decreasing order of susceptibility, are harmed by the larvae: cauliflower, cabbage, broccoli, Brussels sprouts, rutabagas, radishes, turnips, rape, turnip rape, and wild mustard (Beirne, 1971). Attacks by the larvae may stunt the growth of plants or even kill them. A young plant may be killed by a single maggot boring into the main root, but it takes many

maggots to kill a larger plant (Neilson and Arrand, 1959). Plants attacked as seedlings or soon after transplanting are usually severely damaged and often die because the combination of larval feeding and subsequent rotting destroys the entire root system. Attacks by the first generation of this insect, when the plants are growing rapidly, often destroy the plants, or, if not, result in wide scars and cause the roots to become hard and woody (Beirne, 1971). The tunnels made by the maggots in the upper part of radish roots make them unmarketable.

According to Smith (1927b) to prevent confusion, a description of the life cycle of D. radicum is best started with the adult. The adults are about 7 mm long, active, greyish-brown flies. They resemble the house fly although they are slimmer and slightly smaller. The male is smaller, darker, more bristly than the female, and with the tip of the abdomen more broadly rounded (Brittain, 1927; Smith, 1927b).

Adult emergence begins when soil temperatures reach 16 C and ceases if the temperatures rise above 21.1 C (Coaker and Finch, 1971). However, my recent work showed that the adults could emerge at 28 C, probably due to local temperature adaptation. Adults of the first generation emerge in late April and May from the overwintering puparia. Six to eight days after they emerge, the females begin to lay eggs in crevices in soil and on the underside of soil crumbs, usually within 5 cm of the host plant (Hughes and Salter, 1959). The females are able to lay their first batch of eggs (40-60) when provided with a source of nutritive carbohydrate. Under laboratory conditions, they usually feed on carbohydrate on the 2nd and 3rd days after

emergence, mate on the 4th day, and begin oviposition on the 5th or 6th day (Coaker and Finch, 1967). To lay the 2nd and subsequent batches of eggs, their diet must include both carbohydrates and quantities of the ten amino acids that have been found to be essential for growth (House, 1954; Friend, 1956) and reproduction (Dimond et al., 1956) in other insects. When not fed on proteinaceous materials, under field conditions, flies of the first generation laid an average of 63 eggs/female (Finch, 1971). Under laboratory conditions and fed on honey solution and yeast, however, up to 165 eggs were recorded from one female (Swailles, 1961).

According to Hawkes (1968), adult feeding occurs mostly in the morning and movement and oviposition in the early afternoon. A contact chemostimulus is necessary before the females can lay eggs. This stimulus from the host plant brings the flies into contact with plant leaves, when oviposition behavior of the gravid females on the soil starts (Coaker and Finch, 1971). The most favorable substrates for oviposition are those with a particle size of 0.60-1.75 mm which provide the maximum number of suitable cracks (Traynier, 1967).

The eggs of D. radicum are white, 0.93-1.02 mm long on average and 0.3 mm broad, and elongate with one side convex and the other slightly concave (Miles, 1952; Varis, 1967). They are normally laid singly and mainly in the soil around the stems of cruciferous plants but under some conditions, they are laid on the heads of cauliflowers (Smith, 1927b) and on Brussels sprouts (Brooks, 1951; Way and Murdie, 1965; Coaker, 1967). They are susceptible to desiccation due to the open-mesh structure of the

shell (Coaker and Finch, 1971). Their average viability is 86-96% under both field and laboratory conditions, with maximum hatch obtained when eggs are kept on a wet surface or at RH of 90% or more (Gibson and Treherne, 1916). The incubation period of the eggs depends on temperature and varies between 2 and 14 days; the threshold temperature for incubation was 7.2 C, and temperatures in excess of 35 C are lethal to eggs (Coaker and Finch, 1971)

The larvae of D. radicum are white, tapering, legless maggots with the head reduced and deeply retracted into the broad prothorax. There are three larval instars which can be differentiated by the size of their mouthparts (Miles, 1952). Young maggots start feeding soon after hatching. They cause damage by first feeding on the secondary roots then mining and burrowing in the main roots (Miles, 1931). Although few newly-hatched maggots fail to reach a host plant, many die trying to become established and mortality can be as high as 73% (Abu Yaman, 1960), but mortality during the other instars is normally low (Abu Yaman, 1960; Hughes and Mitchell, 1960). The larval stage usually lasts 19-32 days (Schoene, 1916; Smith, 1927b; Varis, 1967), but it may be reduced by the lack of food or water (Schoene, 1916) or extended if temperatures are low (Smith, 1927b).

Larval damage is usually followed by soft rot of the roots caused by bacteria (Coaker and Finch, 1971). Larvae normally become contaminated with bacteria from the soil, decaying plant material, or from contaminated egg shells. Erwinia spp. were predominant amongst the pathogens isolated from puparia bred on

rutabaga; Pseudomonas spp. and Aerobacter spp. from puparia bred on Chinese cabbage (Doane and Chapman, 1964). Although the larvae transmit decay-causing bacteria to healthy tissues, aiding in the development and spread of the rot, the association between the larvae and the bacteria is coincidental, not obligatory (Doane and Chapman, 1964).

The puparia of D. radicum are about 8 mm long and 3 mm broad, dark brown, sub-elliptical, with smoothly rounded sides and can be identified by the hardened protuberances which are the remains of larval posterior tubercles (Miles, 1952). They are formed from the integument of fully-grown larvae in the soil near the host plant roots, or inside the roots if the soil is very dry. The pupa forms within the puparium. In the field, pupal stages of the early generations last from 12 to 18 days (Schoene, 1916), whereas those of the later generations, which overwinter in the soil, may last from 5 to 10.5 months (Schoene, 1916; Varis, 1967) and in exceptional cases 2 years (MacLennan, 1922). Temperatures of 16.1-21.7 C are suitable for pupal development, but those over 33.5 C are lethal (Harris and Svec, 1966). The pupae can be induced into diapause, and photoperiod, temperature, and light intensity are the external factors responsible; but diapause can be terminated by exposure to low or high temperatures (Hughes, 1960; Read and Welch, 1962; Read, 1969).

The number of generations of the cabbage maggot in a year depends on prevailing climatic conditions and varies from one in the northern U.S.S.R. to five in parts of the U.S.A. and Canada

(Coaker and Finch, 1971). There are two or three generations per year in British Columbia (Neilson and Arrand, 1959).

Many natural factors affect the population of the cabbage maggot. Key factor analysis showed that "misadventure" of the larvae between hatching and the second moult is a key factor, and pupal parasitism is considered a stabilizing factor (Mukerji, 1971). Eggs are preyed on by trombidid mites (Lowry, 1914), ants (Schoene, 1916) and carabid and staphylinid beetles (Wishart, et al., 1956). Total egg and early larval mortality is caused mainly by four factors: predation, reduced viability, drought effects on newly-hatched larvae, and the failure of the latter to find the host plant (Hughes and Salter, 1959). Of these, egg predation accounted for over 90% of the losses in the first generation (Hughes, 1959). Larvae are preyed on by ants (Schoene, 1916), beetles (Abu Yaman, 1960; Read, 1962; Coaker, 1965) and other anthomyiid larvae (Smith, 1927b). The cynipid, Idiomorpha rapae (Westw.), which lays its eggs on the first- and second-stage larvae, is the only hymenopterous parasite of major importance and has been recorded from more than 60% of the pupae in some samples (Wishart, 1957). A few anthomyiids (Schoene, 1916; Read, 1958) and cordylurids (Read, 1958) have been reported as predators of the adult stage of the cabbage maggot. The fungus Entomopathora muscae Cohn kills the adults, whereas the fungus Strongwellsea castrans Batko only sterilizes them (Smith, 1927a).

The early attempts to control the cabbage maggot were based on one or several cultural, physical and non-insecticidal

methods. However, none of these methods was reliable and practical on a large scale (Coaker and Finch, 1971).

It was not until the 1940s that organochlorine insecticides were developed for controlling the cabbage maggot (Bonnemaison, 1965), and truly practical and reliable control of this pest was achieved (Wright, 1954). The main disadvantage of using organochlorine insecticides over a long term is that they are toxic to the natural enemies of the cabbage maggot (Morris, 1960; Read, 1960; Pimentel, 1961; Coaker, 1966; Hassan, 1969). Furthermore, this insect has developed resistance to some of them, such as DDT, aldrin, dieldrin and gamma-BHC in several countries (Harris, et al., 1962; Howitt and Cole, 1962; Coaker, et al., 1963). Alternative insecticides for controlling this insect have been found among the organophosphorus and carbamate compounds (Bonnemaison, 1965). These compounds are less persistent than the organochlorines and are selective at application rates that are not toxic to predatory beetles (Mowat and Coaker, 1967). Early application of these compounds can help brassica crops withstand some injury without serious reduction in yield (Coaker and Finch, 1965). However, they rarely give complete control of the larval damage on crops where there are several months between treatment and harvesting. It is well-known that all these insecticides have caused more or less harmful pollution in the environment.

Other methods have been used or suggested for controlling this insect. These included growing resistant host plants (Swailles, 1959; Pond et al., 1962; Matthewman and Lyall, 1966) and releasing sterilized flies (Swailles, 1966).

In Canada, this insect is said to be the most important vegetable pest in Newfoundland and Labrador, the most important single factor limiting turnip production in New Brunswick, one of the main factors limiting high quality rutabaga production in Alberta, and the most serious pest of cabbage and cauliflower in British Columbia, where it is a critical factor in the production of early cabbage in the coastal region (Beirne, 1971). In British Columbia, the control of this pest has mainly relied on insecticides, such as Lorsban 4E, Dasanit SC, Dasanit 15G, Diazinon 50 WP, and Basudin 50 WP (Savage, 1989). When these products are used properly losses in plant stand and crop value could be reduced to a minimum of 5% or less; without effective chemical control losses would be 50% or higher (Sweeney, pers. comm.). Chlorpyrifos, an organophosphate, has been proved to be more effective than diazinon, and chlorpyrifos 4E formulation is now being used almost exclusively for the control of this pest in B.C. (Mackenzie, et al., 1987). The main disadvantage of the insecticides mentioned is that they may cause phytotoxicity, especially under hot weather or dry conditions (Mackenzie, et al., 1987).

Some steinernematid and heterorhabditid nematodes have been considered as biocontrol agents of many insect pests (Poinar, 1979). These two groups of entomopathogenic nematodes belong to the families Steinernematidae and Heterorhabditidae respectively in the order Rhabditida. The life cycle of these nematodes includes the egg, four juvenile stages, and the adult stage.

The third-stage juveniles of these two groups, called the infective juveniles (IJJs), are ensheathed by the second-stage

juvenile cuticles, and contain cells of the symbiotic bacteria Xenorhabdus spp. in their intestines. These cuticles are shed while the IJs are penetrating through the midgut wall or the exo-skeleton (in the case of heterorhabditids) into the haemocoel of a host (Bedding and Molyneux, 1982). The ensheathing cuticle may play an important role in protecting the IJs from adverse environmental conditions, such as desiccation. The IJs are the only free-living stage in the environment. They do not feed, but by living off their energy reserves they are able to live for long periods under favorable conditions of humidity, temperature, and oxygen.

The life cycles of these two nematode families are as follows. Steinernematid IJs actively or passively enter a host through its natural openings--mouth, anus or spiracles (Poinar, 1979; Mracek, et al., 1988). They then actively penetrate through the midgut wall or tracheae into the hemocoel and release the symbiotic bacteria through the anus (Poinar, 1966). The bacteria then multiply rapidly, cause septicemia and kill the host within 24-48 h. The immature nematodes feed on the growing bacterial population and necrotic host tissues, and develop to male and female adults. These first generation adults are usually much larger than the adults of subsequent generation(s) (Wouts, 1980). Steinernematids produce only amphimictic (i.e. with males and females) generations. The giant females mate with the smaller males immediately after molting. Eggs develop rapidly and initially are laid in the host's haemocoel by the females 24 to 48 h after mating (Poinar, 1979). Later batches of eggs hatch inside the mother's uterus and

subsequently fill the body of the dead female nematode prior to bursting out into the insect's haemocoel. Under condition of high humidity, new IJs leave the host's cadaver and enter the environment, where they wait for a new host. Several generations can occur in a single host, depending on conditions and the host's size. My work showed that Steinernema feltiae requires 10 to 14 days to complete its life cycle in late instar larvae of the greater wax moth, Galleria mellonella, at 22 C, whereas Heterorhabditis spp. required a few days longer.

The life cycle of heterorhabditids is similar to that of steinernematids but with some key differences. In addition to entering a host through natural openings, heterorhabditid IJs may directly penetrate through the exo-skeleton at the relatively soft intersegmental membrane regions by using a tooth-like structure located dorsally close to the mouth (Bedding and Molyneux, 1982). After entering the hemocoel of a host, the IJs all develop into hermaphroditic females which, in turn, produce an amphimictic generation. The males and females of this generation mate and produce a new generation of hermaphroditic individuals which leave the host cadaver and enter the soil environment, and search for a new host.

The bacteria associated with steinernematids and heterorhabditids belong to the genus Xenorhabdus (Thomas and Poinar, 1979), which was originally described as Achromobacter (Poinar and Thomas, 1965). They have been found only in the intestines of the vector nematodes or their insect hosts (Poinar, 1966; Poinar, et al., 1977; Akhurst, 1982a), and are not known to occur free in the environment.

X. nematophilus (Thomas and Poinar, 1979) is associated with the steinernematids. Based on phenotypic (Akhurst, 1983; 1986a,b,c) and electrophoretic studies (Hotchkiss and Kaya, 1984), this species is further split into four subspecies, namely nematophilus, bovienii, poinarii, and beddingii. The heterorhabditids are associated with X. luminescens (Thomas and Poinar 1979), a bacterium named after the bioluminescent appearance of insect cadavers infected with this nematode-bacterial complex. Both species of bacteria occur as primary and secondary forms. The primary form is isolated from the IJs and newly-infected insects, and it supports greater nematode production than the secondary form in both in vivo and in vitro cultures (Akhurst, 1980). A wide range of microorganisms that may be present in insect hosts or in in vitro systems and decrease nematode production are inhibited by the primary form, but not by the secondary form (Akhurst, 1982b). Both forms are equally infectious when injected intrahemocoelically into insect hosts (Akhurst, 1980). Moreover, the secondary bacteria adhered to the haemocytes of G. mellonella to a greater extent than did the primary bacteria (Dunphy and Webster, 1984), suggesting that the former suppressed the host's defensive system better than did the latter.

The relationship between these nematodes and the bacteria is one of classic mutualism (Poinar and Thomas, 1966). The nematodes are able to selectively ingest and retain Xenorhabdus cells even in the presence of other microbes and selectively retain cells of the primary form when both primary and secondary forms exist in a single host (Akhurst, 1980). When introduced

into the hemocoel of a insect host, axenic nematodes (i.e., without their associated bacteria) are generally able to kill the host and may develop to adults, but reproduction is rare or poor (Poinar and Thomas, 1966). As few as 1-3 cells of Xenorhabdus may be pathogenic to G. mellonella (Poinar, 1979; Milstead, 1979). However, the bacteria are unable to enter an insect without the nematode vector (Poinar and Thomas, 1966; Milstead, 1979).

Steinernematids and heterorhabditids have been commercially produced and marketed on a small scale for the control of insect pests (Woodring and Kaya, 1988). They can be reared in vivo or in vitro. For in vivo production, G. mellonella is often used as a host because it is large, widely available, easily reared, and very susceptible to nematode infection. Up to 200,000 S. feltiae (Dutky, et al., 1964) and 350,000 H. bacteriophora IJs (Milstead and Poinar, 1978) have been harvested from one last-instar G. mellonella. For in vitro production, potato mash (McCoy and Glaser, 1936, cited by Woodring and Kaya, 1988), ground veal pulp (McCoy and Girth, 1938, cited by Woodring and Kaya, 1988), and dog food (Hara, et al., 1981) have been used as culturing media. Bedding (1981, 1984) has developed a commonly-used technique in which a chicken offal medium on a porous foam substrate is used and thus a great number of IJs may be economically produced.

The defensive mechanisms exhibited by insects against the invading nematodes have been summarized by Poinar (1979). These include host escape, cellular responses, and humoral responses. R. Bedding (cited by Akhurst, 1986d) observed that scarabaeid

larvae possess sieve plates over spiracles, and that they are able to push nematodes away from their mouths with the anterior legs, suggesting that they use structural or behavioral measures to avoid or escape nematode invasion. Other morphological barriers, such as the puparia and egg shells of D. radicum, can block nematodes from entering. Encapsulation and melanization of H. heliothidis, which is a cellular or humoral response, have been observed in the haemolymph of adult cockroaches (Zervos and Webster, 1989). Dunphy and Webster (1987) found that the lipid component of the epicuticle of infective juvenile S. feltiae strain DD136 prevents encapsulation of the nematodes in vivo by haemocytes of G. mellonella larvae. This is considered to be advantageous to the nematodes in that it ensures an optimum condition in the haemocoel for development of the associated bacteria. An insect may harm the nematodes indirectly by offering defense against their symbiont bacteria, which the nematodes release after entering. The total haemocyte counts in the haemolymph of G. mellonella increased after the introduction of X. nematophilus nematophilus, and by 6 h after injection more than 92% of the bacteria were cleared by haemolymph; but this marginal defense appeared to be overcome by rapid bacterial multiplication (Dunphy and Webster, 1984). Introduction of monoxenically cultured IJs of S. feltiae Mexican strain also resulted in an increase in the total haemocyte counts in G. mellonella, but their symbiont bacteria were not harmed to the same extent as X. nematophilus nematophilus (Dunphy and Webster, 1986).

Environmental conditions, abiotic and biotic factors, influence the survival, development, infectivity, and behavior of these nematodes. They are particularly sensitive to desiccation, and dry out and die rapidly when exposed to low humidity on soil and foliar surfaces. Susceptibility to desiccation has often been cited as a main reason for inadequate control of target insect pests in bio-control programs. Steinernematids sprayed on plant foliage in the field may survive for about an hour (Welch and Briand, 1961a). Activity and infectivity of S. feltiae IJs peaked at soil humidity of 25-40% (weight of water/weight of soil) (Kondo and Ishibashi, 1985). Some species (e.g., IJs of the agriotos strain of S. feltiae) are able to survive RH levels well below the permanent wilting point of plants (about pF 4.2), when exposed to gradual desiccation under laboratory conditions (Simons and Poinar, 1973). Various antidesiccants have been tested to prolong nematode survival at low humidities (Welch and Briand, 1961a; Webster and Bronskill, 1968; Bedding, 1976; Kaya and Reardon, 1982; MacVean, et al., 1982; Shapiro, et al., 1985). Among these antidesiccants, Folicote and paraffin oil are the most promising agents tested to date (Bedding, 1976; Shapiro, et al., 1985)

Acceptable temperature ranges for survival, infection, and development, vary with nematode species and strains (Kaya, 1977; Molyneux, et al., 1983; Molyneux, 1984, 1985, 1986). S. feltiae survives at -10 C to 35 C (Schmeige, 1963) and is infective at 9 C to 33 C (Dutky, et al., 1964; Molyneux, 1986). Its optimum growth and reproduction occurs at 23 C to 28 C (Kaya, 1977; Molyneux 1986). In general, steinernematids (with 4-14 C lower

threshold) remain active at lower temperatures (Molyneux, 1984, 1986) and over a broader range of temperatures (Molyneux, 1986) than do heterorhabditids (with 10-16 C lower threshold).

Observations of nematodes mass-reared on G. mellonella in our lab reveal that Heterorhabditis spp. take longer to complete their life cycles than do Steinernema spp. at room temperature (22 C).

Molyneux (1986) showed that parasitization of L. cuprina and G. mellonella occurred in a temperature range greater than that permitting nematode development and reproduction. This would seem to imply that nematodes may penetrate and kill insects at low or high temperatures, but that temperature conditions permitting life cycle completion are more stringent.

Temperature may affect the nematodes indirectly through its effect on their symbiont bacteria. Maximum yield of H. heliothidis in monoxenic culture occurred at 30 C, which is also the optimum temperature for the growth of its associated bacterium, X. luminescens (Dunphy and Webster, 1989).

Since the nematodes are obligate aerobes, their oxygen requirements have been found to be temperature dependent (Burman and Pye, 1980).

Less is known about the influence of other abiotic factors on the nematodes. Solar radiation and UV light are detrimental to S. feltiae (Gaugler and Boush, 1978; Gaugler, et al., 1989). Some UV protectants are able to extend the longevity of the IJs (Gaugler and Boush, 1979). According to Gaugler (1981), factors such as pH, photoperiod and salinity are unlikely to influence the nematodes seriously in most habitats. In fact, S. feltiae

and H. heliothidis were cultured best respectively at pH 6.0 and pH 6.5-7.5, and their associated bacteria, X. nematophilus nematophilus and X. luminescens, grew best respectively at pH 6.5 and pH 6.5-7.5 (Dunphy and Webster, 1989).

Little is known about the effect of biotic antagonists on these nematodes in soil. But natural enemies of the nematodes do exist. The nematophagous fungi, Arthrobotrys oligospora and Monacrosporium ellipsosporum, are able to use a sticky knob to attack, infect and digest some steinernematids and heterorhabditids within 72 h (Poinar and Jansson, 1986b). Infection of the nematodes with endoparasitic fungus (Drechmeria coniospora) has been reported by Poinar and Jansson (1986a). Predatory mites such as Gamasellodes vermivorax, and a collembolan, Hypogastura scotti, feed, develop, and reproduce on the IJs of S. feltiae and H. heliothidis, resulting in a loss in the efficacy of these nematodes against G. mellonella (Epsky, et al., 1988). Other nematode-parasitic microorganisms, such as viruses and bacteria, also exist (Mankau, 1980). During the storage of the nematodes used for the present study, contamination of a nematode suspension with bacteria and predatory mites was observed, and this was, at least partially, responsible for the decline of nematode populations. All these natural enemies may pose a direct threat to the nematodes applied in soil for the control of insect pests.

The IJs are able to disperse both vertically and horizontally in soil and are able to successfully infect a host, although different nematodes may vary in their predominant direction of migration (Moyle and Kaya, 1981; Georgis and Hague,

1981; Georgis and Poinar, 1983a,b,c). The IJs are also dispersed in the environment by infected hosts (Timper, et al., 1988) and rain or irrigation water. Soil texture and soil moisture potential affect their movement and their subsequent infectivity. They travel more easily and cause a higher level of parasitism of insects in soil with low than with high clay content, although differences in migration and parasitism exist between different nematode species in the same kind of soil (Molyneux and Bedding, 1985). My observations suggest that it is desirable to keep soil sufficiently moist to allow the IJs to stand on their tails and wave, as described by Kondo and Ishibashi (1986), because this behavior appears to be necessary for effective contact with the host.

Apart from the accidental contact with a host, the IJs actively seek out the host apparently by responding to chemical and physical cues. The following substances have been shown to be attractive to the IJs of S. feltiae: CO₂ (Gaugler, et al., 1980), defined chemical and bacterial gradients (Pye and Burman, 1981), excretory products such as aqueous washes from insects (Schmidt and All, 1978) and fecal components (Schmidt and All, 1979), and heat (Byers and Poinar, 1982). Larval G. mellonella have been found to be attractive to the infective juvenile S. feltiae DD136 strain (Pye and Burman, 1978). In addition, the IJs are attracted to basic pH (8.6-9.7) but repelled by a pH of 2.5 and by 7.5 mM ammonium (Pye and Burman, 1981).

The IJs of these two genera are able to infect more than 200 insect species from several orders and some other arthropods (Poinar, 1975, 1979). They have no known toxicity to plants

(Poinar, 1979). Since they are unable to survive or develop and reproduce under the relatively high temperature conditions maintained in homoithermic animals, such as mice and chicks, and the hives of honey bees, these nematodes have been shown to be safe to these animals (Poinar, et al., 1982; Kaya, et al., 1982). The US Environmental Protection Agency has exempted steinernematid and heterorhabditid nematodes from registration and regulation requirements (Gaugler, 1988). However, many beneficial insects such as parasitoids may be susceptible to nematode infection during at least part of their life cycles (Kaya, 1985), although such impact is generally assumed to be slight if the control program of target insects is carefully designed and carried out.

Some field applications of nematodes against soil-inhabiting insect pests have been successful. For example, application of infective juvenile H. heliothidis T327 strain to the soil resulted in up to 100% parasitized larvae of the black vine weevil (Otiiorhynchus sulcatus) on potted yew, raspberries and grapes in nurseries, and over 87% on potted cyclamens and strawberries (Bedding, 1981). However, other applications of nematodes have yielded poor control of some insect pests. For example, spraying the nematodes of DD136 strain on potato foliage yielded insignificant reductions in the population of the Colorado potato beetles (Leptinotarsa decemlineata) (Welch and Briand, 1961a). Desiccation (Welch and Briand, 1961a) and extremes of temperature (Gaugler, 1988), which would inactivate (if not kill) the nematodes, are considered to be the main causal factors.

In the present study, two insect-parasitic nematode species, namely S. feltiae Filipjev (Wouts, et al., 1982) (= Neoaplectana feltiae Filipjev; = N. carpocapsae Weiser; = N. agriotos Veremchuk; = N. belorussica Veremchuk; = N. chresima Glaser, McCoy & Girth) and H. heliothidis (Khan, Brooks and Hirschmann) 1976 (= Chromonema heliothidis Khan, Brooks, and Hirschmann, 1976), were investigated for their ability to control the cabbage maggot. The object of my study was to investigate the infectivity and efficiency of these nematodes against this insect in both the laboratory and the field, and to evaluate this control method. Some aspects of the behavior of the nematodes in the presence of this insect and its host plants were investigated in detail.

CHAPTER 2: GENERAL MATERIALS AND METHODS

1. The Cabbage Maggot, *D. radicum*

a. Source

The cabbage maggots used in this project were reared from puparia that were obtained originally from J. Whistlecraft at the Agriculture Canada Research Center, London, Ontario.

b. Rearing *D. radicum*

(1). Preparation of adult food

The food for *D. radicum* adults was composed of the following ingredients by weight: sugar (4 parts); skim milk powder (4 parts); soya flour (1 part); yeast (1 part); and yeast hydrolysate (1 part). First, the sugar and skim milk powder were ground in a pestle separately. Then, they were mixed with the other ingredients and ground again into a fine powder, which was stored in a closed bottle at room temperature for use.

(2). Setting up a colony and collecting the eggs

About 200 to 300 puparia were maintained in a Petri dish (100x15 mm) with a moist filter paper until they were ready to emerge. Upon emergence, the adults were released in a cage (40x25x25 cm) (Fig. 1). They were provided with about 5 grams of the powdered food on a Petri dish lid. The food had been sprinkled with water using a 1/2 liter garden sprayer before the dish lid containing the food was placed in the cage for them to feed. Water was provided in a flask fitted with cotton wicks.

An oviposition site was made by placing a Petri dish lid with a thin layer of autoclaved sand of about 1.0 mm particles and a piece of fresh rutabaga (5x5x0.5 cm) in the cage.



Fig. 1. Maintaining a colony of Delia radicum at 20-25 C, with a photoperiod of 16 h a day. A: adult food; B: water; C: an oviposition site.

The adults were maintained at 20-25 C and 50-70% RH. Photoperiod was 16 h per day.

Oviposition began on the sand when the flies were about one week old. After the sand was removed from the cage, the eggs were either collected by a flotation method, if needed for experimental use, or left with the sand if needed only for maintaining the insect population.

To obtain newly-hatched larvae, fresh eggs were kept on a moist piece of filter paper in a Petri dish where they began to hatch after 3 days at 16.5-24.5 C.

(3). Starting a culture for larvae and collecting puparia

A fresh rutabaga of commercial size bought from a supermarket was washed with soap and water and placed in a plastic container of 15x15x10 cm (but could be of any size that could hold the rutabaga) by cutting the bottom portion of the rutabaga so that it sat firmly on the floor of the container. Autoclaved sand was poured into the container around the rutabaga until the sand was about 2.5 cm from the top rim of the container (Fig. 2(a)). Distilled water was added until the sand was evenly moist without any free water.

The newly-laid eggs in dry sand were poured onto the moist sand around the rutabaga. Any exposed eggs were covered with a thin layer of moist sand. To avoid overcrowding, 200 to 400 eggs were used for one rutabaga, depending on the size of the latter. Such a culture was kept moist by sprinkling distilled water onto the sand 2 or 3 times a week.

About two weeks after the eggs were introduced into the culture, larvae began to leave the rutabaga and move into the

(a) :



(b) :



Fig. 2. A culture for rearing larval and pupal Delia radicum at 20-25 C: (a) newly-laid eggs have been introduced into the moist sand and (b) after about 2 weeks, mature larvae coming out from a rutabaga and pupating in the sand.

sand where they pupated 1 or 2 days afterwards (Fig. 2(b)). When most of the larvae had pupated, the rutabaga was removed and the sand containing the puparia was poured into a container of water. A strainer was used to collect the floating puparia, which were kept in a Petri dish with a piece of moist filter paper until the adults emerged.

Third-instar larvae were obtained by cutting a rutabaga open 10-12 days after the eggs were introduced into the culture. They were kept overnight on moist filter paper in a Petri dish. Those damaged during collection usually died during the night. Healthy ones were used for the control experiments. Mature larvae were collected from the sand as soon as they left the rutabaga.

To obtain newly-formed puparia, mature larvae were collected from the sand, washed with water, and kept overnight in a dish with moist filter paper at room temperatures. Most of them had pupated by the following day.

2. The Nematodes

a. Source and rearing

Two nematode strains of S. feltiae, namely DD136 (Poinar, 1967) and Breton (Byers and Poinar, 1982), one strain of H. heliothidis, namely T327 (Bedding, et al., 1983), and two strains of undescribed species of Heterorhabditis, namely NC162 (Curran, 1989) and A13-5, were used in this study. DD136 and Breton were obtained from Dr. G. O. Poinar (University of California at Berkley) in the early 1980s. T327, isolated from Tasmania, Australia, was obtained from Dr. R. A. Bedding (CSIRO, Hobart) in 1987. NC162 was isolated from North Carolina, USA and

obtained from Dr. W. Brooks in 1983. A13-5 was isolated from British Columbia, Canada in 1984. Now the taxonomic status of T327 and NC162 is in doubt, and they are believed to belong to H. zealandica (Curran, pers. comm., see Appendix 16).

These five strains of nematodes were reared in larval G. mellonella, collected using the methods of Wouts (1984), and stored in moist, oxygenated sponge in sealed packages at 12 C. The infective juveniles could be stored in this form for up to one year without significant decline in infectivity, even though some mortality of the juveniles occurred. However, they were usually recycled every six months to ensure that vigorous nematodes were used for the behavioral studies.

Prior to being used for behavioral or control tests, the nematodes were removed from the storage incubator and allowed 2-4 h to adapt to the prevailing room temperatures.

b. Counting nematodes

Infective juveniles of the above strains were extracted from storage sponge by rinsing the sponge with distilled water in a container. The resulting nematode suspension was transferred to a plastic tube, in which the nematodes could settle to the bottom within 10-15 min. Then, a nematode suspension of moderate density was obtained by decanting most of the water. The suspension was shaken vigorously, and a sample of measured volume was taken with a 2 or 5 ml pipette. This sample was divided into drops of approximately equal size on a Petri dish lid, and the nematodes in each drop were counted under a low power dissecting microscope. The sum of these counts was the number of nematodes in the known volume. Mean number of

nematodes in the known volume was determined by taking three such samples. Thus, a volume of this suspension containing the required number of nematodes was calculated from the mean number per unit volume.

3. Host Plants of *D. radicum*

Rutabaga (*Brassicae napus* L. var. *napobrassica* (L.), Rchb.), radish (*Raphanus sativus* L.), ball cabbage (*B. oleracea* L. var. *capitata* L.), and kohlrabi (a cross between *B. oleracea* L. var. *capitata* L and turnip, *B. rapa* L.), were used for this project. They are all host plants of *D. radicum*. The first three species were used for laboratory experiments, whereas radish and kohlrabi were used for a field trial. Radish was grown from seeds and rutabaga bought fresh commercially. Ball cabbage and kohlrabi were grown from seeds.

CHAPTER 3: EXPERIMENTS AND RESULTS

A. Selection of Nematode Strains for Controlling the Cabbage Maggot

The object of this study was to test the infectivity and efficacy of S. feltiae strains DD136 and Breton, and Heterorhabditis sp. strains NC162 and A13-5, against D. radicum, and to select the best strain(s) for the control of this insect pest.

According to my own observations (unpublished data), D. radicum does its greatest damage in the third instar, thus it is desirable to control the pest in its first and second instars.

This experiment was conducted using 25 Petri dishes (60x15 mm) which were divided into five groups of five each, and prepared as follows: a piece of moist filter paper (55 mm diam) was placed in each dish, on which was placed a slice of rutabaga (7.3x6.0x1.4 mm), sterilized with alcohol and rinsed with sterilized water, as food for the maggots. To each dish of the first group, approximately 400 nematodes of DD136 in aqueous suspension were added. The same procedure was followed for each of the remaining four nematode strains. Distilled water alone was added to the final group of five Petri dishes for a control. The nematodes were distributed evenly on the filter paper and the rutabaga to ensure contact between the nematodes and the maggots. At this point, ten newly-hatched first instar maggots were put into each Petri dish, seven maggots on the filter paper and three on the rutabaga. The dishes were sealed with Parafilm and maintained at 16.5-24.5 C in the dark.

The dishes were examined every 24 h for insect mortality. Dead maggots were removed and kept in moist Petri dishes for one day to allow any invading nematodes to grow sufficiently to be easily seen. Then, the cadavers were dissected and examined for the presence of nematodes in the haemocoel.

Comparisons of significance were made between treatments and the control, and among treatments by using Student's t test.

Results

Results of this test are summarized in Fig. 3 and Appendix 1. For the first 5 days, there were significant differences ($P < 0.01$) in maggot mortality between the control and the treatments with NC162 and A13-5; but no significant differences between the control and the treatments with DD136 and Breton. For the first 6 days, significant differences existed between the control and the treatments ($P < 0.01$, $P < 0.05$, and $P < 0.01$ for Breton, NC162, and A13-5, respectively) but not for that with DD136. For the first 5 days, NC162 caused much higher mortality of the maggots than did DD136 and Breton ($P < 0.01$); A13-5 caused higher mortality of the maggots than did DD136 ($P < 0.05$), but the difference between A13-5 and Breton was not significant. There were no significant differences between NC162 and A13-5 in maggot mortality and in percentages of dead maggots with nematodes, for the first 5 and 6 days.

Soon after the maggots were placed on the filter paper and rutabaga slices, they tunneled into the rutabaga and fed inside. Moulting began on the 3rd day. By the 4th day, all maggots were second-instar, had nearly consumed the rutabaga, and begun to emerge. Most of the maggots in the treatments had died by the

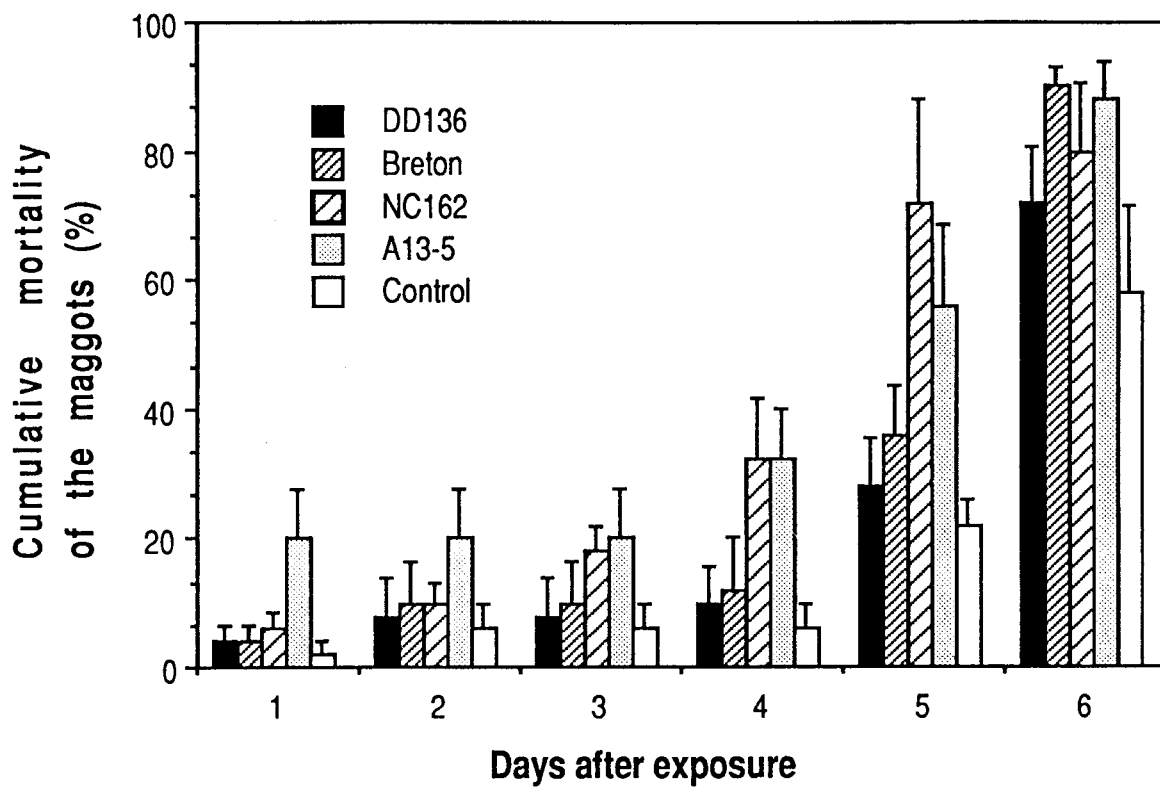


Fig. 3. Day/mean cumulative mortality curves for Delia radicum, exposed to four nematode strains, namely DD136 and Breton of Steinernema, and NC162 and A13-5 of Heterorhabditis, for 6 days, at 16.5-24.5 C. Top bars stand for standard errors (SE) (n=5).

5th or 6th day (Fig. 3). No nematodes were found in the dead maggots from the control.

B. Behavior of the Nematodes in the Presence of Larval and Pupal

D. radicum

Understanding the behavior of the nematodes in the presence of the cabbage maggot is important for choosing the best nematode strain to control this insect and for making the control measures economically viable. Since the most effective nematodes in section A were heterorhabditids, *H. heliothidis* strain T327 was included in later tests. Using the puparia and frozen, dead, third-instar larvae, qualitative and quantitative experiments were done to determine how the nematodes behaved over time and how many responded to the target insects. All experiments were conducted on thin-layer agar in Petri dishes.

1. Qualitative experiments

(1). Experiment with puparia

Newly-formed puparia were surface sterilized with 80% alcohol for 30 s, rinsed five times with sterilized water, and dried on filter paper. Using a water-proof pen, I marked the centers of six Petri dishes (100x15 mm) on the undersides and also the apexes of equilateral triangles with the center of each dish as the midpoint of the triangle. The apexes were 2 cm from the midpoint. Each dish contained a very thin layer of 1.5% Bacto Agar. In each of three dishes, two puparia were placed at the midpoint; for three control dishes, no puparia were added. The dishes were sealed with Parafilm and incubated at 22-23 C for 2 h. Then, using a fine, mounted hair, three infective-stage

juveniles of strain T327 were placed at the marked apexes of each of these six dishes, one nematode at each apex. At 21 C, using ILFOSPEED grade 5 paper on a 4x5 enlarger (make: OMEGA; type: PRO-LAB) with the techniques modified after Ward (1973), three photographs were taken, one at each of 5 min, 15 min, and 30 min after the nematodes were introduced. Subsequently, seven more photographs of each dish were taken at intervals of 30 min, for a total of ten. The number of nematodes which were present at each target of puparia was determined by tracing nematode tracks on agar such as those shown in Fig. 4, and recorded in Appendix 2.

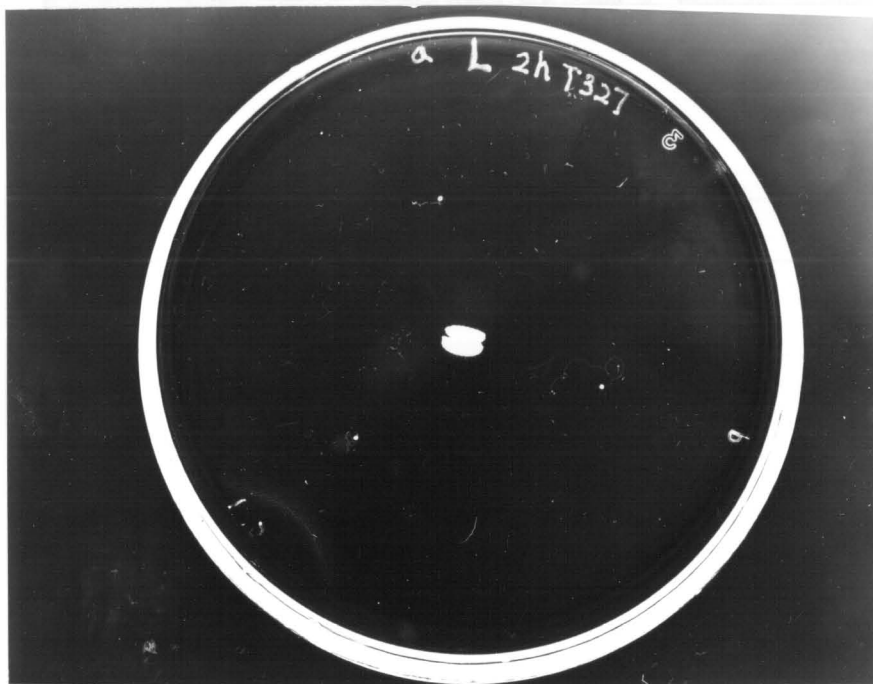
Later, 10 more replicates with puparia were set up using the same methods and under similar conditions. However, the controls were omitted this time since the results of the three control replicates described had shown that nematodes of strain T327 moved randomly on agar in the absence of target insects.

(2). Experiment with larvae

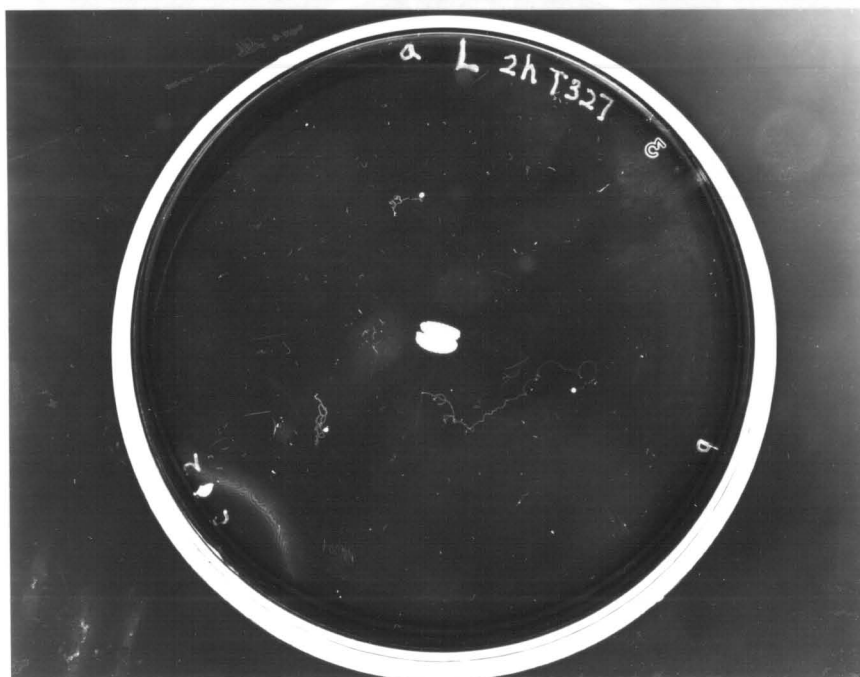
After two-week-old, third instar larvae of D. radicum were surface sterilized, rinsed, and dried using the methods described above, they were incubated at 22-24 C for 1 h. Then, they were killed by freezing for 8 min, after which they were removed and thawed for 30 min. Two such larvae were placed at the center of each of three dishes prepared as in the last experiment. No larvae were placed in another three dishes, the controls. All dishes were sealed with Parafilm and incubated at 22-24 C for 2 h. At 21 C, three infective juveniles of strain T327 were introduced into each dish, and photographs were taken at intervals, as described above. Nine more replicates including

Fig. 4. Tracks showing the orientation of infective juvenile Heterorhabditis heliothidis strain T327 (at three per dish) towards frozen, dead, third-instar larval Delia radicum (at two per dish), over time, at 21 C, on thin agar: (a) after 5 min and (b) after 15 min.

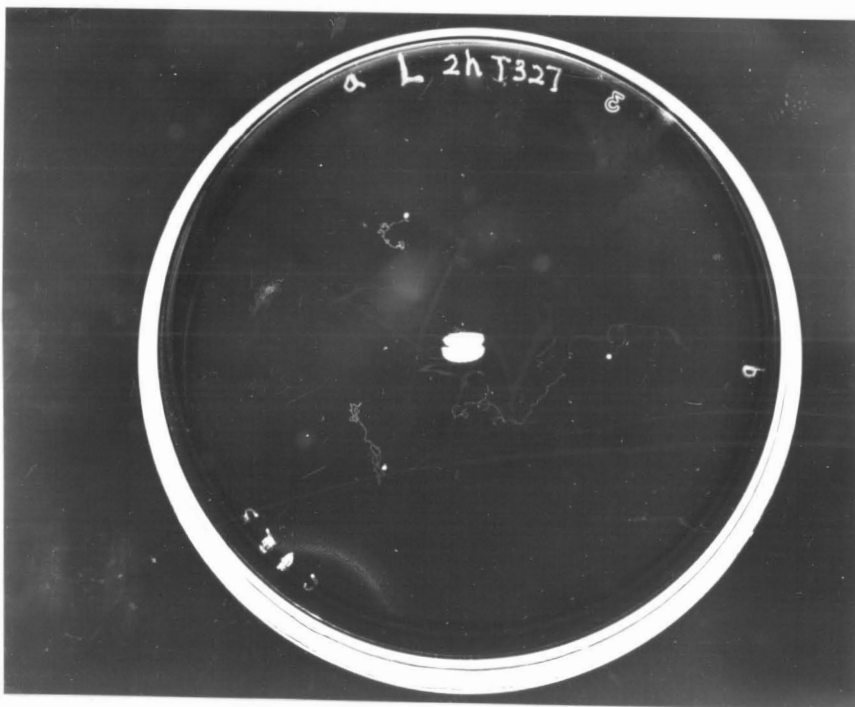
(a) :



(b) :



(c) :



(d) :

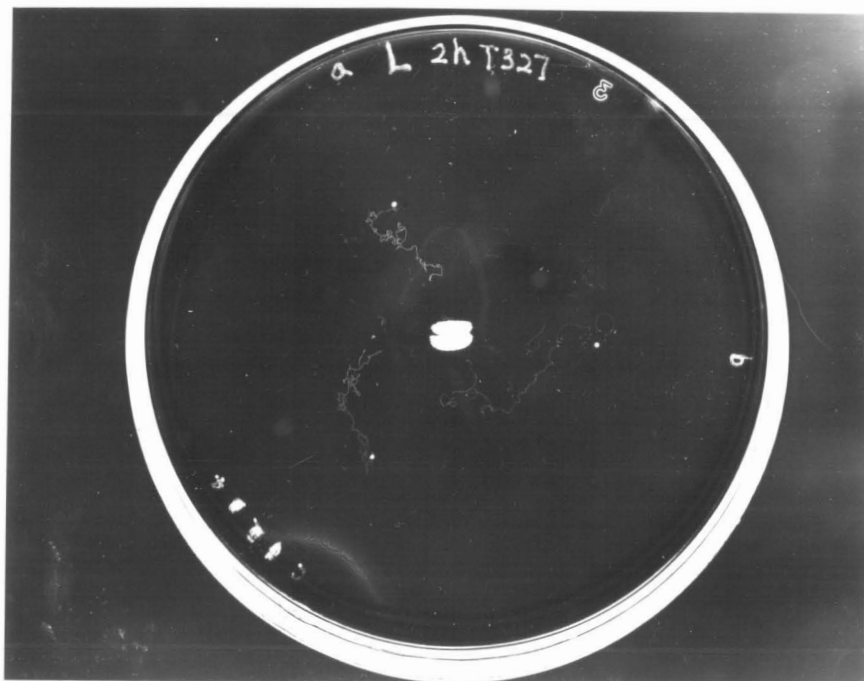
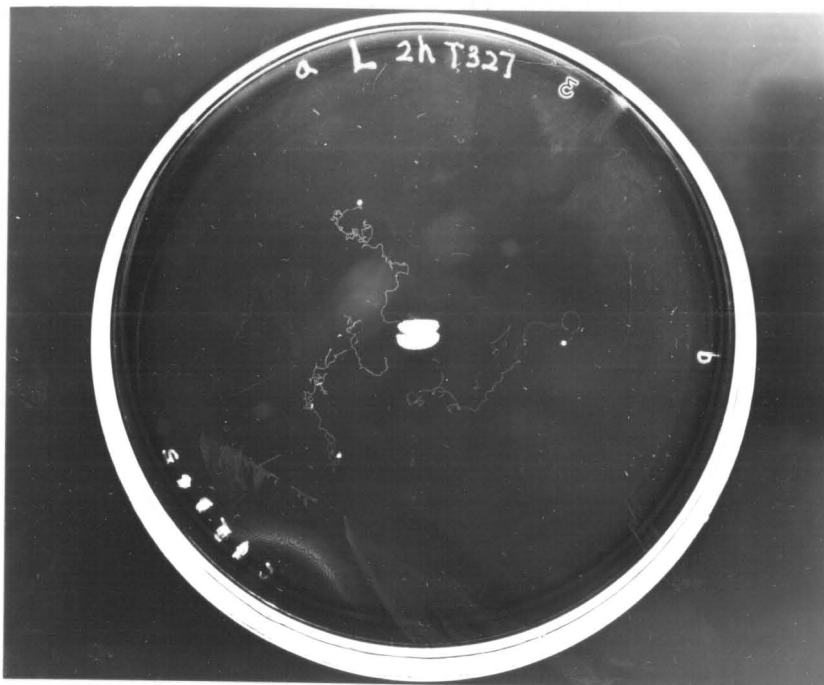


Fig. 4. Legend as on page 32a but (c) after 30 min and (d) after 1 h.

(e) :



(f) :

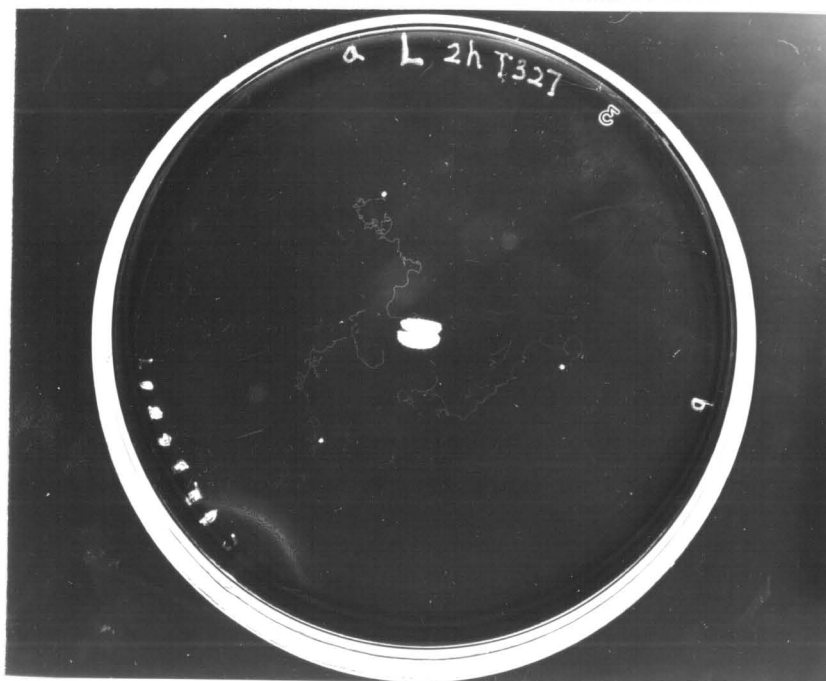


Fig. 4. Legend as on page 32a but (e) after 1.5 h and (f) after 4 h.

the controls were set up on three different days under similar conditions.

The numbers of nematodes which were present at the larvae were determined (Appendix 3) by tracing the tracks left by nematodes on the agar.

RESULTS

Soon after the nematodes were placed on the agar, they began to move closer to the insect targets over time (Fig. 4). Some of the nematodes reached the puparia and the larvae within 15 min, and this number of nematodes increased with time (Figs. 5 and 6). Within 4 h, about 33% and 64% of the nematode inocula reached and remained at the puparia and the larvae, respectively (Figs. 5 and 6), although some of the nematodes first reached the targets, then left and returned (Appendixes 2 and 3). The nematodes in the controls moved randomly.

2. Quantitative experiments

(1). Experiments with puparia

a. Using two-month-old infective nematodes

Newly-formed puparia, obtained as described earlier, were washed with distilled water, dried on paper towels, and transferred to filter paper (Whatman #1) discs of 7 mm diam on 1.5% Bacto Agar (5 ml in each 100x15 mm Petri dish). In each of five agar Petri dishes, the puparia were arranged as shown (Fig. 7). Paper discs without puparia were the controls. The dishes were sealed with Parafilm and incubated at the prevailing room temperature for 1 h. Then, to each of the five dishes, using a fine, mounted hair, from 200-400 infective juveniles of H. heliothidis strain T327, which had been stored at 12 C for two

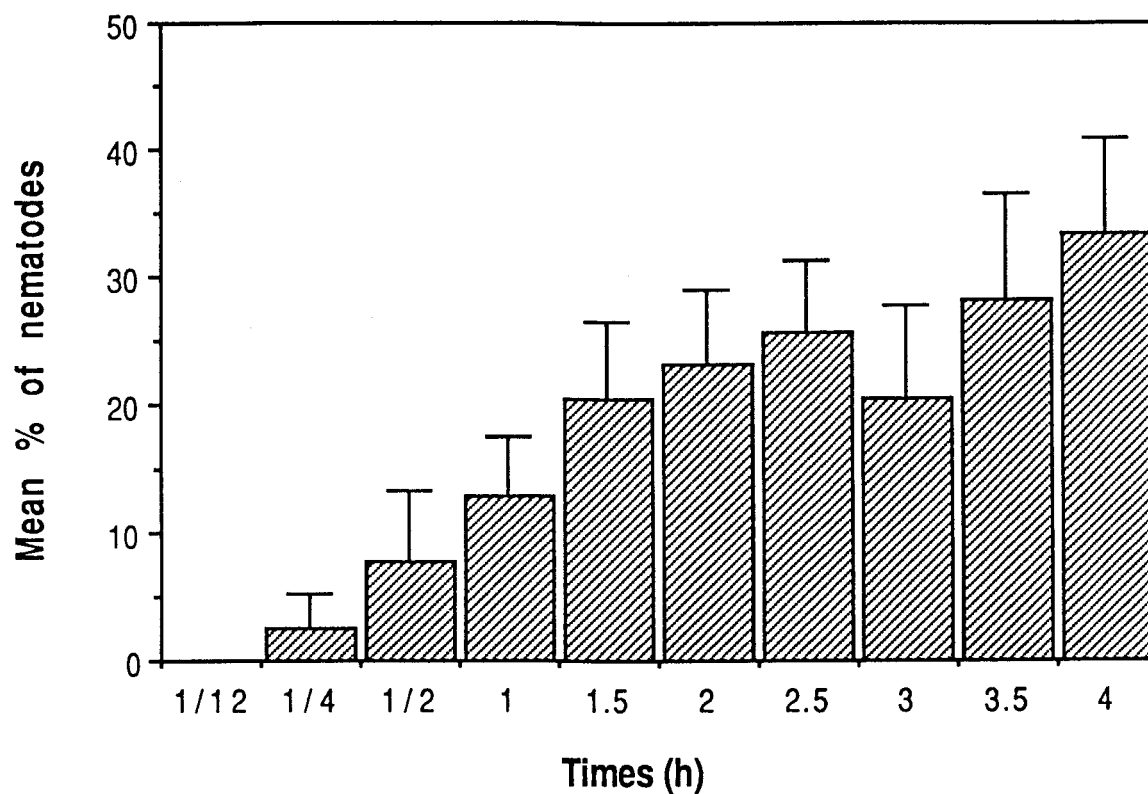


Fig. 5. Mean percentages of introduced, infective juvenile Heterorhabditis heliothidis strain T327 (at three per dish), which reached newly-formed puparia of Delia radicum, over time, at 21 C. Top bars stand for standard errors (SE) (n=13).

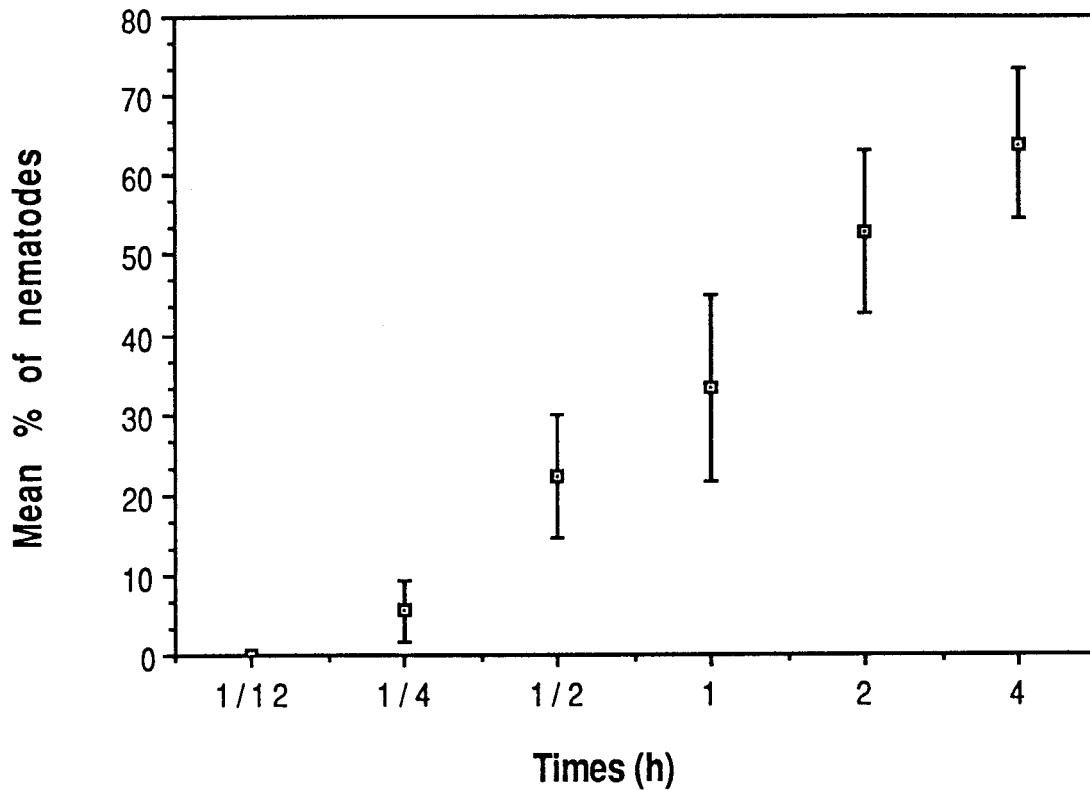


Fig. 6. Mean percentages of introduced, infective juvenile Heterorhabditis heliothidis strain T327 (at three per dish), which reached frozen, dead, third-instar larval Delia radicum, over time, at 21 C. The bars stand for standard errors (SE) (n=12).

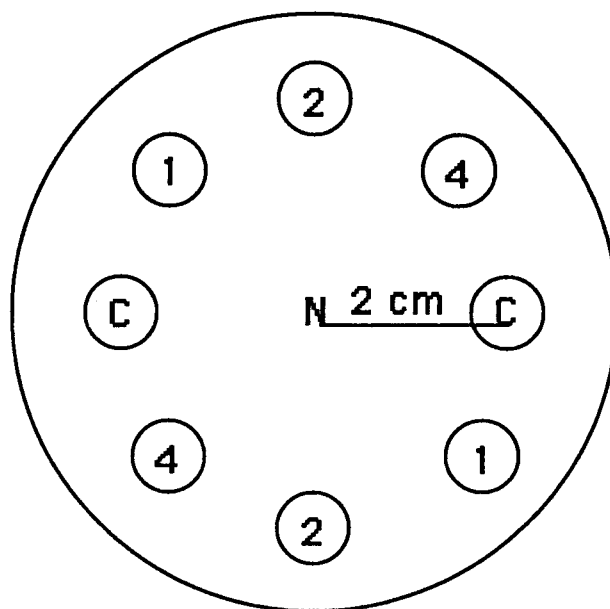


Fig. 7. Arrangement of infective juvenile

Heterorhabditis strain T327 or NC162, and of pupal or larval Delia radicum on filter paper discs, on agar in a Petri dish. N= nematode introduction point; 1, 2, & 4= numbers of insects; C= control.

months, were placed at the center of the agar plate. The dishes were resealed and incubated at 24-24.5 C.

Four hours after the nematodes were introduced, the puparia and paper discs were removed with forceps and washed separately with water. Later, the nematodes in the washes were counted under a dissecting microscope. The nematodes present in and on the agar beneath the paper discs also were counted immediately after the puparia and paper discs were removed.

b. Using newly-harvested and one-month-old infective nematodes

To test if the nematodes oriented to a new insect host immediately after they were collected from their in vivo culture, and if there was any difference in this behavior between different nematode strains, two experiments, one involving strain T327 and the other strain NC162, were set up using the methods described above and under similar conditions. Newly-formed puparia of D. radicum were used as targets, and there were five replicates of each experiment.

Since the results of this experiment with newly-harvested juveniles of strain T327 showed that these nematodes did not orient to the puparia, another experiment was conducted using those T327 nematodes which had been collected from the same culture and stored at 12 C for one month. This test was done under similar conditions and divided into two subtests, of which one was ended 4 h and the other 24 h after the nematodes were introduced.

Comparisons between the treatments and the control, and among the treatments, were made using Student's t test.

(2). Experiments with larvae

Infective juveniles of strains T327 and NC162, and larval D. radicum and G. mellonella, were used for the following experiments to see if these two nematode strains responded differently from each other and if these two insect species influenced T327 differently.

In an experiment using five Petri dishes for each nematode strain and D. radicum larvae, the latter were collected, sterilized, and killed using the methods described for the qualitative experiments. In another experiment, larvae of about equal weight of D. radicum and G. mellonella, were washed (not sterilized) with distilled water to remove the dirt, killed by freezing, then thawed using the methods described previously, and arranged on filter paper discs on agar in each of ten Petri dishes as shown (Figs. 7 and 8). Infective juveniles of strain T327 were introduced into the Petri dishes after 2 h, and the dishes were sealed with Parafilm and incubated at 20-23.5 C. Twenty-four hours after the nematodes were introduced, using the methods described above, the nematodes which had reached the targets were counted. The total of nematodes at the targets included those that had entered the larvae, which were dissected 4 days after being removed from the target site.

RESULTS

In experiments with two-month-old T327 nematodes and newly-formed puparia of D. radicum, the nematodes were attracted by the puparia (Fig. 9 and Appendix 4). There were significant differences between the control and the treatments in the percentage of nematodes which reached the targets ($P < 0.05$

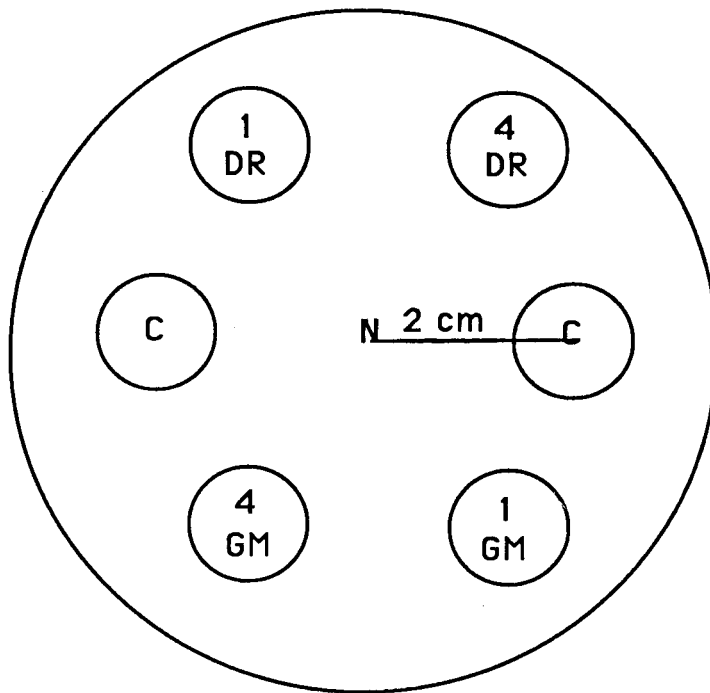


Fig. 8. Arrangement of larval Delia radicum (DR) and Galleria mellonella (GM) on filter paper discs, and of infective juvenile Heterorhabditis heliothidis strain T327, on agar in a Petri dish. N= nematode introduction point; 1 & 4= numbers of insects; C= control.

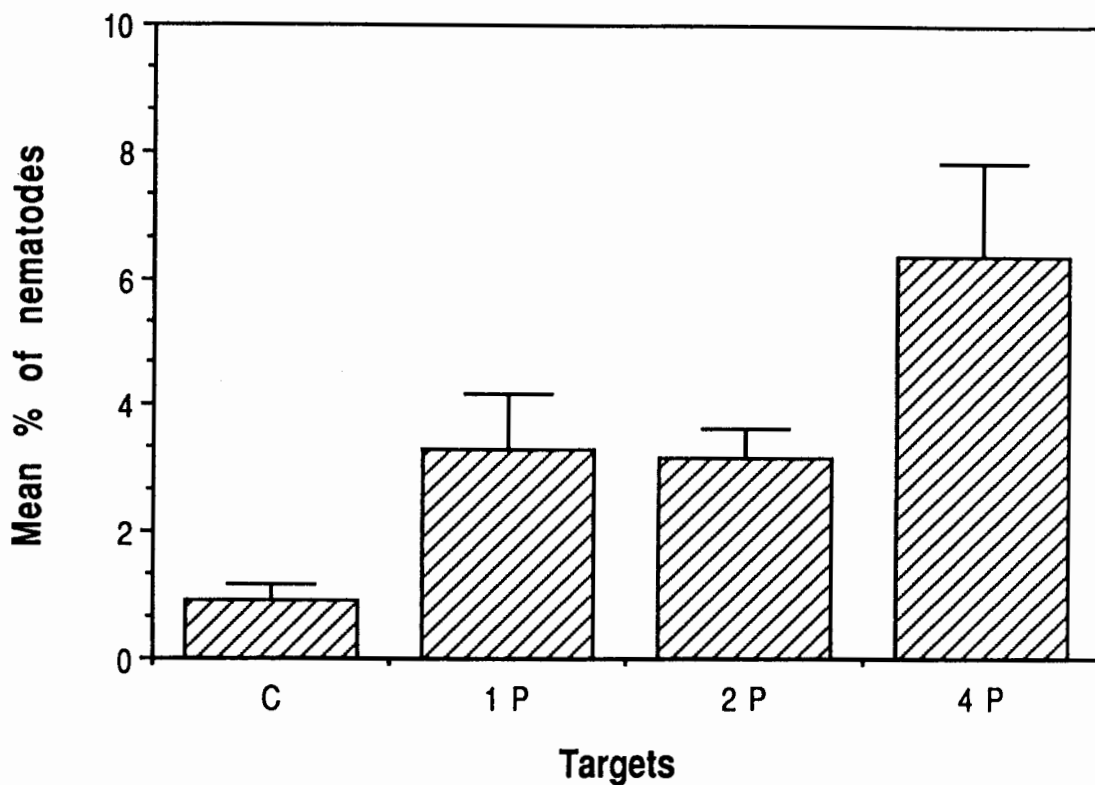


Fig. 9. Mean percentages of introduced, two-month-old, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of one (1 P), or two (2 P), or four (4 P) newly-formed puparia of Delia radicum, and targets of the control (C), within 4 h, at 24-24.5 C. Top bars stand for standard errors (SE) (n=10).

between control and one puparium; $P < 0.01$ between control and two and four puparia). However, there was no tendency for a greater number of puparia to attract more nematodes, because the differences among the treatments were not significant.

Newly-harvested infective juveniles of strain T327 appeared not to respond to the puparia, because 4 h after about 200 nematodes were introduced, only three nematodes had reached three targets of two puparia and one nematode had reached one target of four puparia. No nematodes were present at the remaining targets. However, newly-harvested infective juveniles of strain NC162 were attracted to the puparia (Fig. 10 and Appendix 5). This is shown by the significant differences in percentages of introduced nematodes which reached targets of puparia and the controls ($P < 0.05$ for one puparium and control; $P < 0.01$ for two puparia and control; and $P < 0.001$ for four puparia and control). Moreover, there was an apparent tendency for multiple puparia to attract more nematodes than did a single larva ($P < 0.05$ for two and one puparia; $P < 0.01$ for four and two puparia; and $P < 0.001$ for four and one puparia).

Unlike the newly-harvested infective juveniles of strain T327, those that had been stored for one month after being collected from the same culture were attracted to the puparia, even though significantly ($P < 0.001$) more nematodes reached targets of only four puparia than those of the controls 4 h after the nematodes were introduced (Fig. 11 and Appendix 6(a)). Within 4 h, no significant differences existed between targets with one or two puparia and controls with none, and there was no tendency for a greater number of puparia to attract more

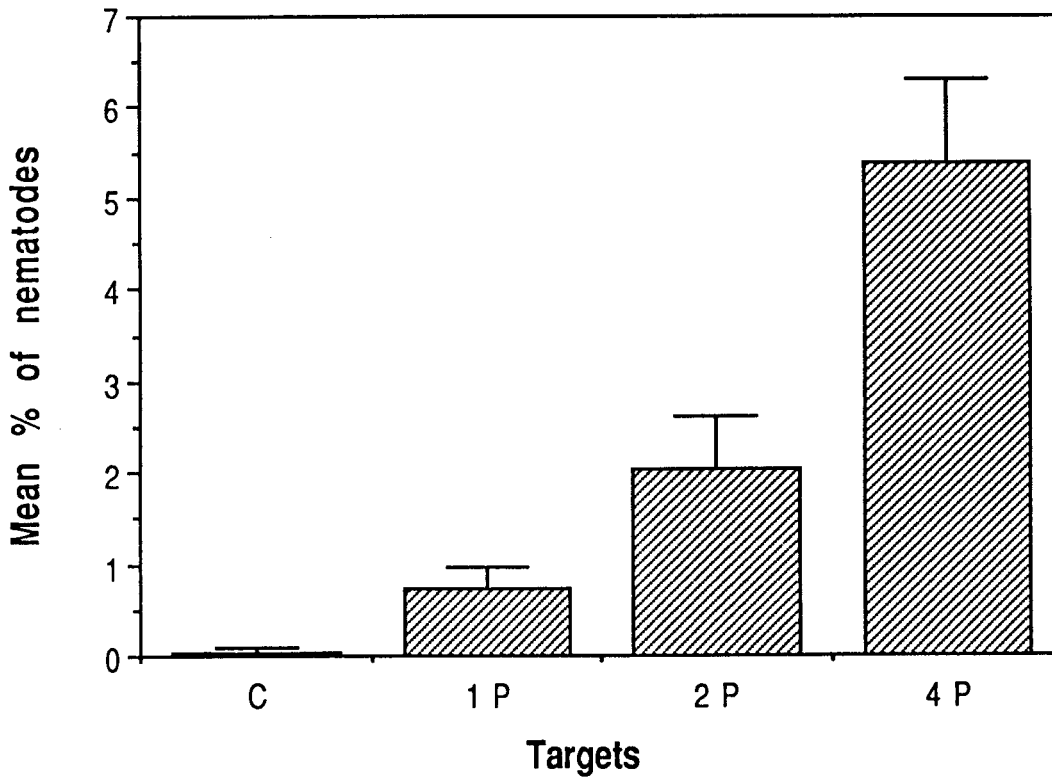


Fig. 10. Mean percentages of introduced, newly-harvested, infective juvenile Heterorhabditis strain NC162, which reached targets of one (1 P) or two (2 P), or four (4 P) newly-formed puparia of Delia radicum, and targets of the control (C), within 24 h, at 23.5-25.8 C. Top bars stand for standard errors (SE) (n=10).

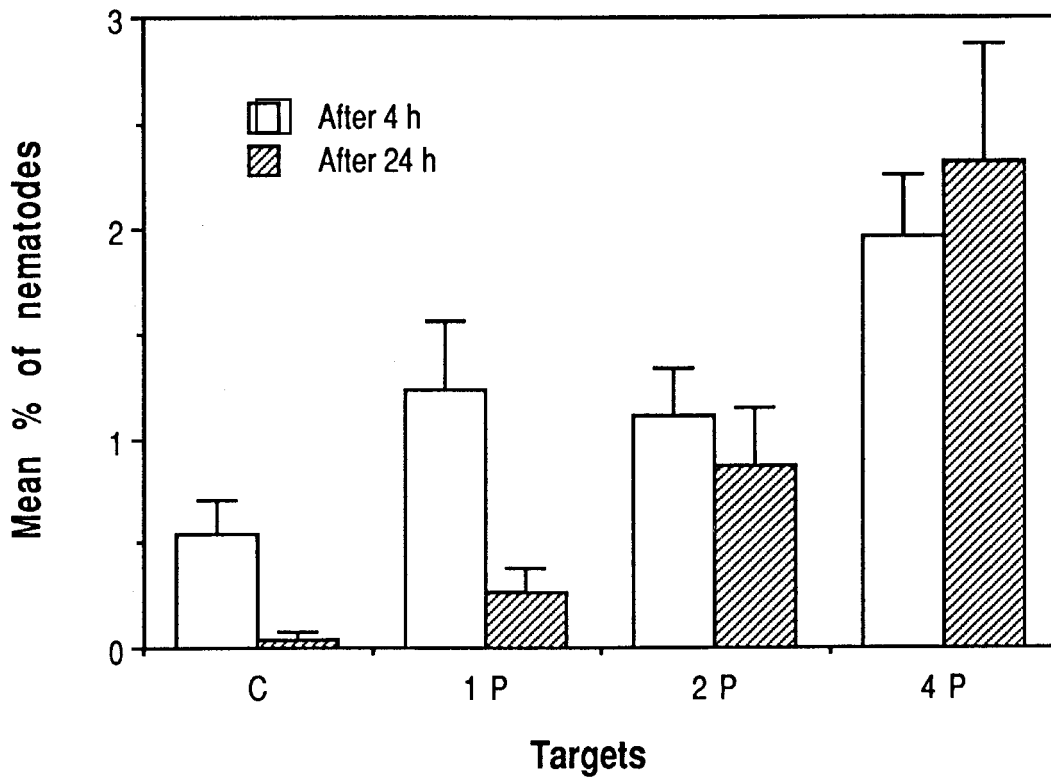


Fig. 11. Mean percentages of introduced, one-month-old, infective juvenile *Heterorhabditis heliothidis* strain T327, which reached targets of one (1 P), or two (2 P), or four (4 P) newly-formed puparia of *Delia radicum*, and targets of the control (C), after 4 h and 24 h, at 23.5-25 C and 23.3-25.1 C, respectively. Top bars stand for standard errors (SE) (n=10).

nematodes (Fig. 11). However, such a tendency occurred when the nematodes were introduced for 24 h ($P < 0.05$ between two puparia and one, and between four and two; $P < 0.01$ between four and one), and the differences between targets of puparia and those of the controls were significant ($P < 0.05$ between one, two puparia and control; $P < 0.01$ between four puparia and control) (Fig. 11 and Appendix 6(b)).

In experiments with both strains and D. radicum larvae, the larvae were attractive to the nematodes. Moreover, there was an apparent tendency for multiple larvae to attract more nematodes of both strains than did a single larva (Fig. 12). In the experiment with T327, there were significant differences between the control and the treatments with the three categories of larvae ($P < 0.05$). Among the treatments, significantly more nematodes were attracted to four larvae than to lower numbers ($P < 0.01$ between four larvae and one; $P < 0.05$ between four larvae and two), but there was no significant difference between one and two larvae. Similar results were obtained in an experiment with NC162 and D. radicum larvae.

Significantly ($P < 0.01$) more T327 than NC162 nematodes were attracted to four larvae, but similar numbers of nematodes of both strains were attracted to two larvae or one (Fig. 12). In addition, nematodes of strain T327 moved more actively on agar than those of NC162 under similar conditions.

In the experiment with T327 on larval D. radicum and G. mellonella, the nematodes began to move randomly immediately after they were placed on the agar. Later, more of them oriented to the insects, but few to the controls. Twenty-four hours after

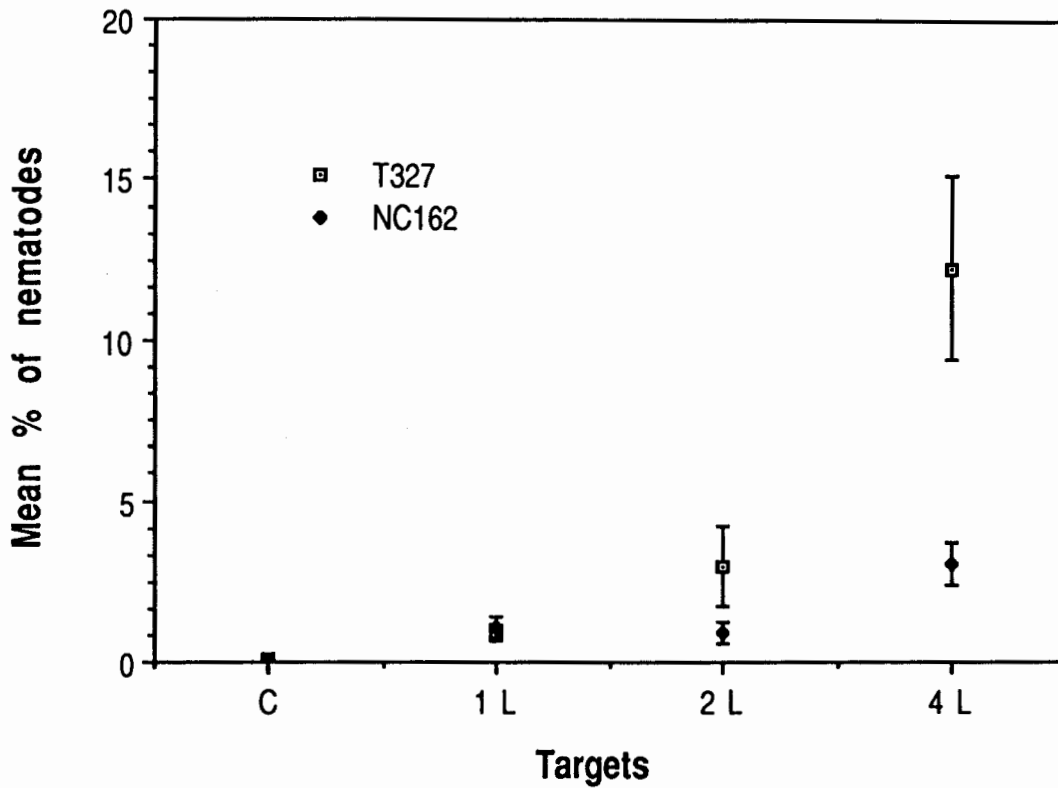


Fig. 12. Mean percentages (\pm SE) ($n=10$) of introduced, infective juveniles of strain T327 or NC162 of Heterorhabditis, which reached targets of one (1 L), or two (2 L), or four (4 L) frozen, dead, 3rd-instar larval Delia radicum, and targets of the control (C), within 24 h.

introduction, most of the nematodes were found crawling on the walls of the dishes.

As with the previous experiments, more nematodes oriented to the targets as the numbers of target insects increased (Fig. 13 and Table 1). Although some nematodes reached the controls soon after they were introduced, none stayed there after 24 h (Appendix 7), and the differences between the treatments with insects and the controls were highly significant (Table 1). However, there were no significant differences in the percentage of nematodes attracted to D. radicum as compared with G. mellonella, although there was a tendency for more nematodes to orient to the former (Fig. 13).

C. Using Selected Nematode Strains to Control the Cabbage Maggot in the Laboratory

Nematodes have been used in the laboratory to control the cabbage maggot at every stage of its life cycle.

1. Eggs

An experiment was done to discover if nematodes kill the eggs of D. radicum, with the aim to control the maggots more effectively before the larvae hatch and tunnel into the host plants.

A thin layer of autoclaved sand was added to each of ten Petri dishes (40x12 mm). Using a pipette, about 5,000 infective juveniles of strain NC162 in aqueous suspension were applied to the sand surface of each of five of the dishes. The surplus water was absorbed with filter paper so that the nematodes could stand on their tails and move easily on the sand. The same

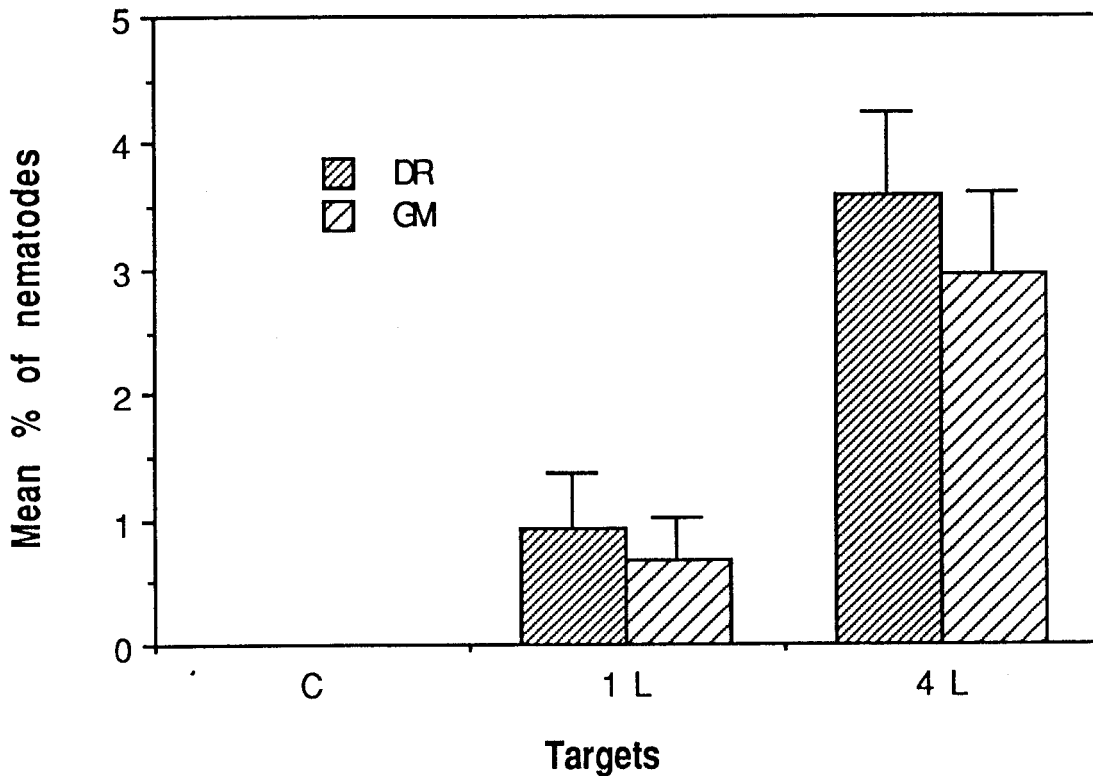


Fig. 13. Mean percentages of introduced, infective juvenile *Heterorhabditis heliothidis* strain T327, which reached targets of one (1 L) or four (4 L) larval *Delia radicum* (DR) and *Galleria mellonella* (GM) (ten replicates for each species), and targets of the control (C) (twenty replicates), within 24 h, at 20-23.5 C. Top bars stand for standard errors (SE).

Table 1. P values from the comparisons of percentages of introduced infective juvenile Heterorhabditis heliothidis strain T327 which reached targets of one or four larval Delia radicum (DR) or Galleria mellonella (GM), and targets of the controls with no insects, within 24 h, at 20-23.5 C.

	1 DR	4 DR	1 GM	4 GM	Control
1 DR		P<0.005	P>0.05		P<0.01
4 DR	P<0.005			P>0.05	P<0.001
1 GM	P>0.05			P<0.01	P<0.005
4 GM		P>0.05	P<0.01		P<0.001

procedure was followed for the remaining experiments in this section. Distilled water alone was added to the remaining five dishes so as to moisten the sand to the same level as that in the five experimental dishes. Then, using two fine brushes (one for the treatment and the other for the control), 15 1- or 2-day-old eggs of D. radicum were placed on the sand in each dish. The dishes were sealed with Parafilm and incubated at room temperature. After 20 h, all the eggs were removed, washed with water, and maintained in clean dishes, each with a piece of moist filter paper. One day later, 70% of the eggs hatched. Then, the larvae and the unhatched eggs were dissected. No nematodes were found in the eggs or larvae from the treatments with nematodes, nor in the controls without nematodes. Many nematodes had been observed crawling on the eggs before they were removed and examined.

2. First-Instar Larvae

It is desirable to kill the newly-hatched larvae quickly, because after they have entered the host plants it may be difficult for the nematodes to gain access to them; control measures are less effective and losses are greater if the nematodes are applied after insect damage occurs. This work was composed of three experiments.

(1). Mortality test of the first-instar larvae of D. radicum

Infective juveniles of strain NC162 were used for this test. Twenty 60x15 mm Petri dishes were prepared as follows: to prevent the maggots from escaping, the ridges on the dish lids were cut off; a piece of filter paper was provided for each dish. Then, using a pipette, about 10,000 nematodes in aqueous

suspension were added to each of ten dishes. To each of the other ten control dishes, only distilled water was added to keep the filter papers about as moist as those in the treated dishes. Using two fine brushes, ten newly-hatched maggots were randomly picked up and placed in the middle of the filter paper of each dish, alternately in the treatments and the controls. The dishes were sealed with Parafilm and incubated in the dark at 20-22 C.

The dishes were examined twice at intervals of 24 h and 48 h after the maggots were introduced. Mortality was determined by touching the maggots with a fine brush. Dead maggots in the treated dishes were removed to clean dishes, but those in the controls were not. Missing maggots were presumed to have escaped and thus considered to be still alive. All the dead maggots were dissected and checked for internal nematodes at the end of this test.

Comparison of the significance of maggot mortality, between the treatment and the control, was made by using Student's t test.

RESULTS

Most of the dead maggots in the treatment contained many nematodes. The nematodes had consumed nearly all the body contents of the maggots leaving only the exo-skeleton, and they were easily seen through the cuticle to be moving inside the cadavers. However, these nematodes failed to develop to adults, probably due to lack of food.

The numbers of dead maggots are recorded in Appendix 8. Mortalities of the maggots from all causes were 20% and 80% in the treatment, 2% and 27% in the control. Differences between

the treatment and the control were thus highly significant ($P < 0.01$). Moreover, 18% and 71% of the maggots were parasitized by the nematodes within 24 h and 48 h, respectively (Fig. 14).

(2). Time for newly-hatched maggots to enter a host plant

The bottom of each of two Petri dishes (100x25 mm) was covered with a thin layer of autoclaved sand, and a radish was placed on the sand. Distilled water was added to keep the sand wet. In one dish, 20 newly-hatched maggots were placed on the sand about 1 cm from the widest part of the radish; in the other dish, 20 maggots were placed on the radish directly. They were all kept in the dark.

RESULTS

All the maggots in both dishes, except two which died, had tunneled into the radishes after less than 6 h.

(3). How effective are the nematodes on newly-hatched maggots within 6 hours?

Infective juveniles of strain NC162 and newly-hatched larvae of *D. radicum*, were used in this test.

Twenty 40x12 mm Petri dishes were divided into four groups of five, with the ridges on the lids removed to prevent the maggots from escaping. To each dish, the following materials were added in sequence: a thin layer of autoclaved sand; about 1,500 nematodes in aqueous suspension; and ten maggots. All the dishes were sealed with Parafilm and incubated at prevailing room temperatures.

After time intervals of 1.5 h, 3.0 h, 4.5 h, and 6.0 h, the maggots in each group of five dishes were removed and washed into clean dishes with water. One to two days afterwards, all

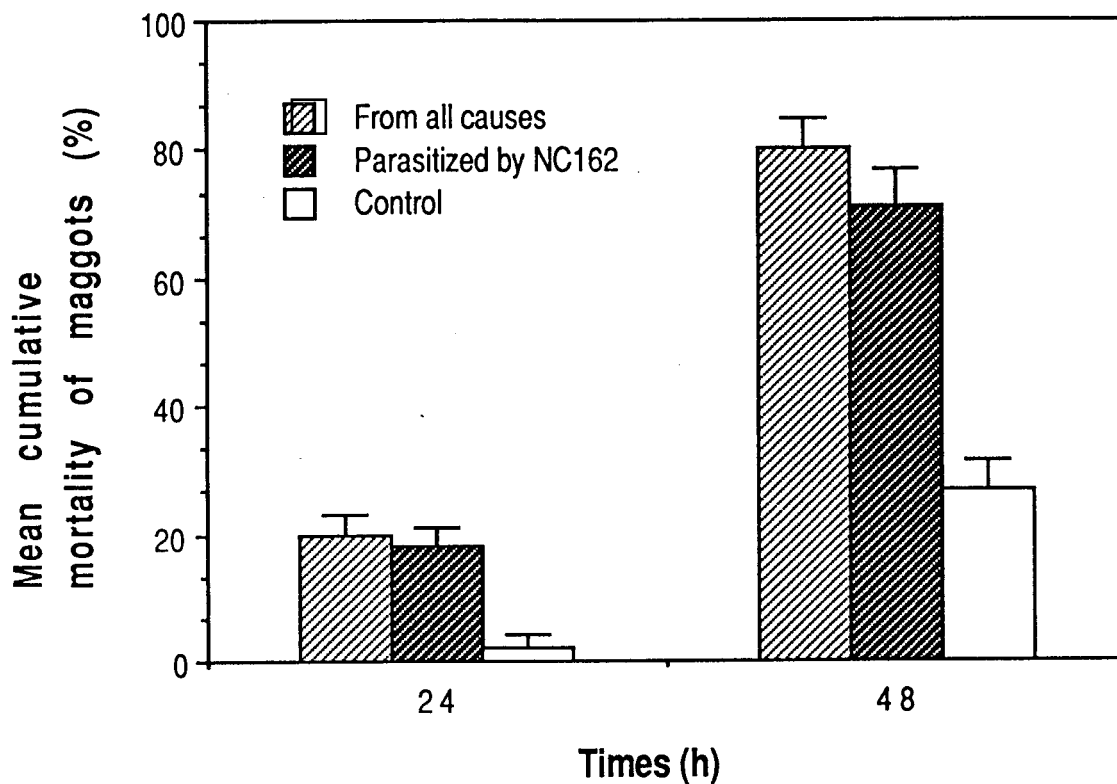


Fig. 14. Mean cumulative mortality of 1st-instar larval *Delia radicum* exposed to infective juvenile *Heterorhabditis* strain NC162, within 24 h and 48 h, at 20-22 C. Top bars stand for standard errors (SE) (n=10).

200 maggots were dissected and checked for the presence of nematodes.

RESULTS

Results are summarized in the following table:

	1.5 h	3.0 h	4.5 h	6.0 h
No. maggots infected	0/50	4/50	4/50	3/50
No. nematodes found	0	7	19	3

As shown above, only 11 out of 200, or 5.5% of the maggots, were infected within 6 h after the nematodes were introduced.

3. Third-Instar Larvae

(1). Comparison of efficacy between strains NC162 and T327

Since strain T327 was attracted by the cabbage maggot, it was necessary to test its efficacy and make comparisons between it and strain NC162 that appeared to be the best strain against this pest in section A. Third-instar larvae of D. radicum are more accessible and easily handled than second-instars, and first-instars had been shown to be ineffectively controlled by the nematodes, therefore third instar larvae were used for this test.

In each of 30 Petri dishes (60x15 mm), 2 ml of autoclaved sand of medium-sized particles was spread in a thin layer. The dishes were divided into three numbered groups of ten. Using a different pipette for each group, about 5,000 infective juveniles of strain NC162 in aqueous suspension were added to

each dish of group 1, 5,000 infective juveniles of strain T327 in aqueous suspension to each dish of group 2, and distilled water alone added to each dish of group 3, which was the control. Then, using forceps, ten healthy maggots were taken at random and transferred to one dish of group 1, ten maggots to one dish of group 2, and another ten maggots to one dish of group 3. Ten maggots were added to each of the remaining dishes with the same procedure. All the dishes were sealed with Parafilm and incubated in the dark at 20-24.8 C.

The dishes were examined every 24 h for 7 days. The numbers of puparia and dead larvae were recorded. A larva was classified as "dead" if it did not move on being touched with a needle. Puparia and dead larvae were removed, washed with water, then kept in clean dishes with moist filter paper for 3-4 days, after which they were dissected and checked for the presence of nematodes. After each examination, all dishes were re-sealed and returned to the dark. Puparia showing adult characteristics were considered to be alive, whereas those without adult characteristics were considered to be dead and dissected for the presence of nematodes.

(2). Using CO₂-pretreated and normal larvae

Mortality of maggots caused by the nematodes in the preceding test was not very high, probably due to their active movement. Thus, if the maggots are slowed or stilled for a time, perhaps more of them could be killed by the nematodes. This hypothesis was tested by treating the maggots with CO₂ before they were exposed to the nematodes.

Healthy, 3rd-instar maggots were divided into two groups. At prevailing room temperatures, the maggots of one group were placed in a flask that was subsequently filled with CO₂ and plugged with a plastic plug for 8 h, whereas those of the other group were incubated on moist filter paper in a Petri dish and they were considered to be normal. Using the methods described above, the CO₂-treated and normal maggots, at ten per dish, were exposed to 5,000 or 500 infective juveniles of strain NC162 on autoclaved sand in (60x15 mm) Petri dish. The maggots in the controls did not receive nematodes. There were five replicates (i.e. five dishes) for each nematode dose and control. The dishes were sealed with Parafilm, incubated at 21.3-22.3 C, and examined for puparia and dead larvae daily at the same time, for 4 days. The puparia and dead maggots were dissected for the presence of nematodes 3-4 days after they had been removed from the dishes.

For both tests, the larval and pupal mortalities between the treatments and controls, and among the treatments, were compared by Student's t test.

RESULTS

In test (1), some maggots were killed by the nematodes of both strains within 24 h. Seven days after commencement, the numbers of dead maggots and puparia were 86 in group 1 (NC162), 70 in group 2 (T327), and 44 in group 3 (control) (Appendix 9). There were significant differences ($P < 0.01$) in the insect mortalities among these three groups (Fig. 15).

All the dead maggots in group 1 contained nematodes (Appendix 9), whereas only 26 out of 50 dead maggots in group 2

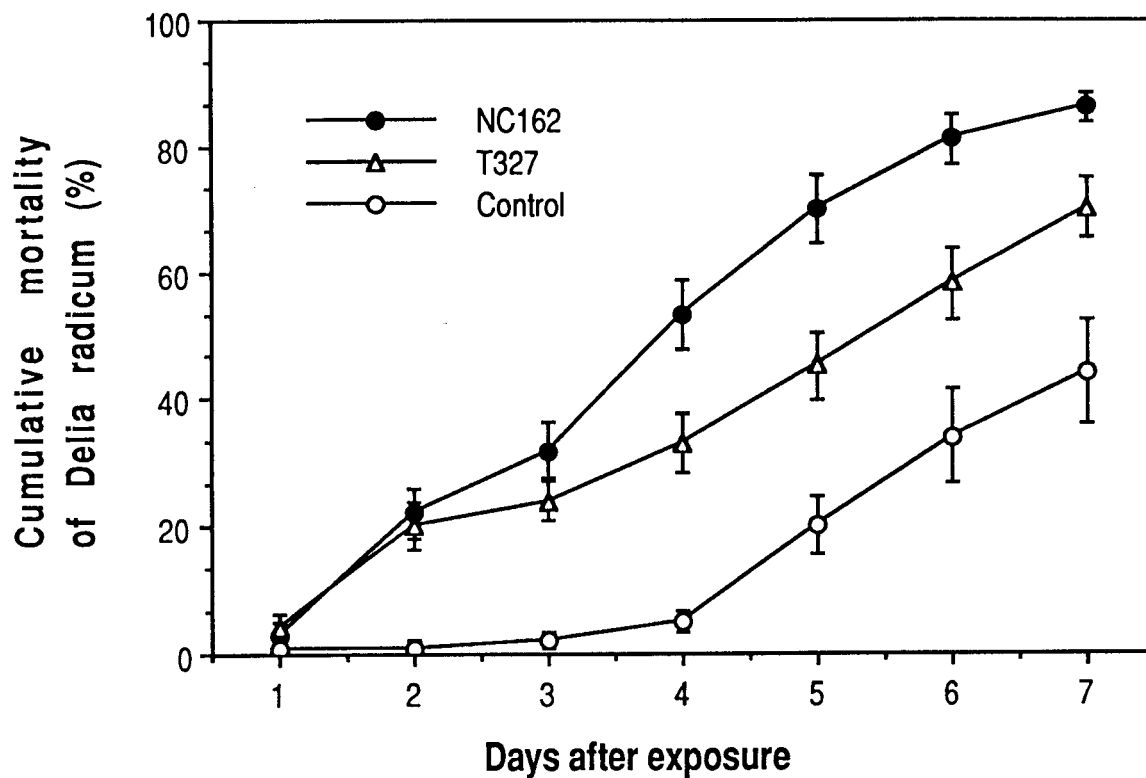


Fig. 15. Mean cumulative mortality of 3rd-instar larval *Delia radicum* exposed to infective juvenile *Heterorhabditis* strain NC162 or T327 for 7 days, at 20-24.8 C. Bars stand for standard errors (SE) (n=10).

contained nematodes, and no nematodes were found in dead maggots in the control group. All the dead puparia in both groups 1 and 2 contained nematodes. No puparia died in the control group.

The insect cadavers in group 1 were full of nematodes of various ages (Fig. 16), but those in group 2 contained many fewer and most of these nematodes failed to develop and died.

In test (2), the results are summarized in Appendix 10. Eight hours of the CO₂ treatment pacified the maggots for less than 1 h, after which they gradually resumed normal movement. During this short period, many nematodes were crawling on the maggots. All dead maggots and puparia from the treatments contained nematodes, whereas those from the controls did not.

As shown in Fig. 17, most of the maggots in the treatments were killed by the nematodes by the 3rd day after exposure, and there was little change in larval mortality between the 3rd and 4th days; few maggots in both controls died, and there were no significant differences in maggot mortality between them. By the 2nd day, virtually all CO₂-treated maggots were killed in the treatment with 5,000 nematodes per dish. When exposed to the same dose of nematodes, significantly more CO₂-treated maggots than normal ones were killed, and mortality increased with a higher dose of nematodes. Within the 1st day after exposure, 500 nematodes killed significantly ($P < 0.01$) more CO₂-treated maggots than did 5,000 nematodes on normal maggots, and within the first 2 or 3 days, similar numbers of maggots were killed by these two doses of nematodes (Fig. 17). The P values from the comparisons within the first 2 or 3 days are listed in Table 2.

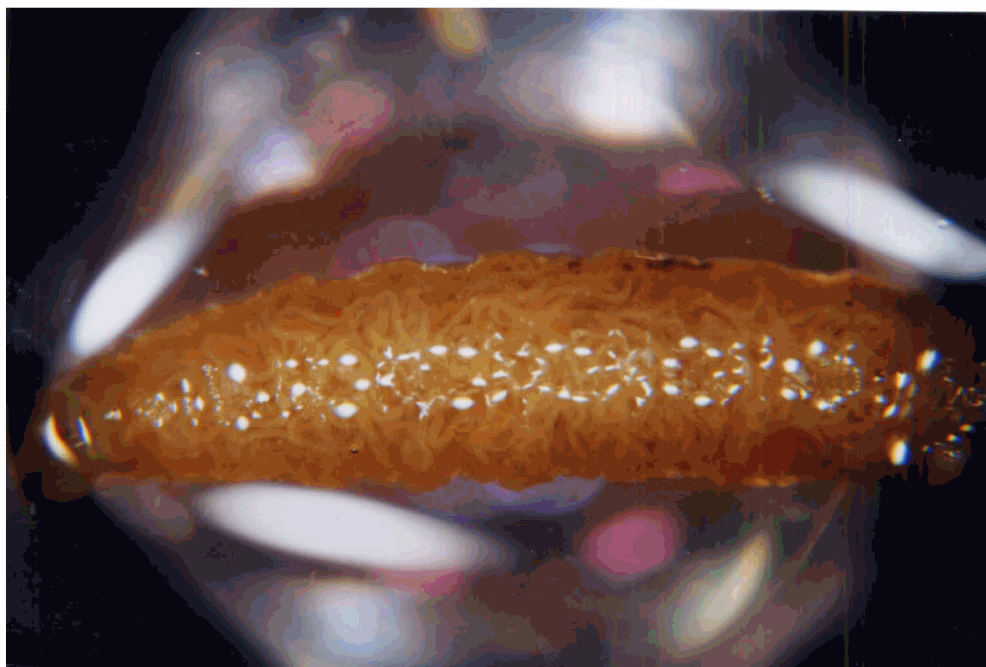


Fig. 16. A 3rd-instar larval *Delia radicum* infected with *Heterorhabditis* strain NC162, containing the developing nematodes.

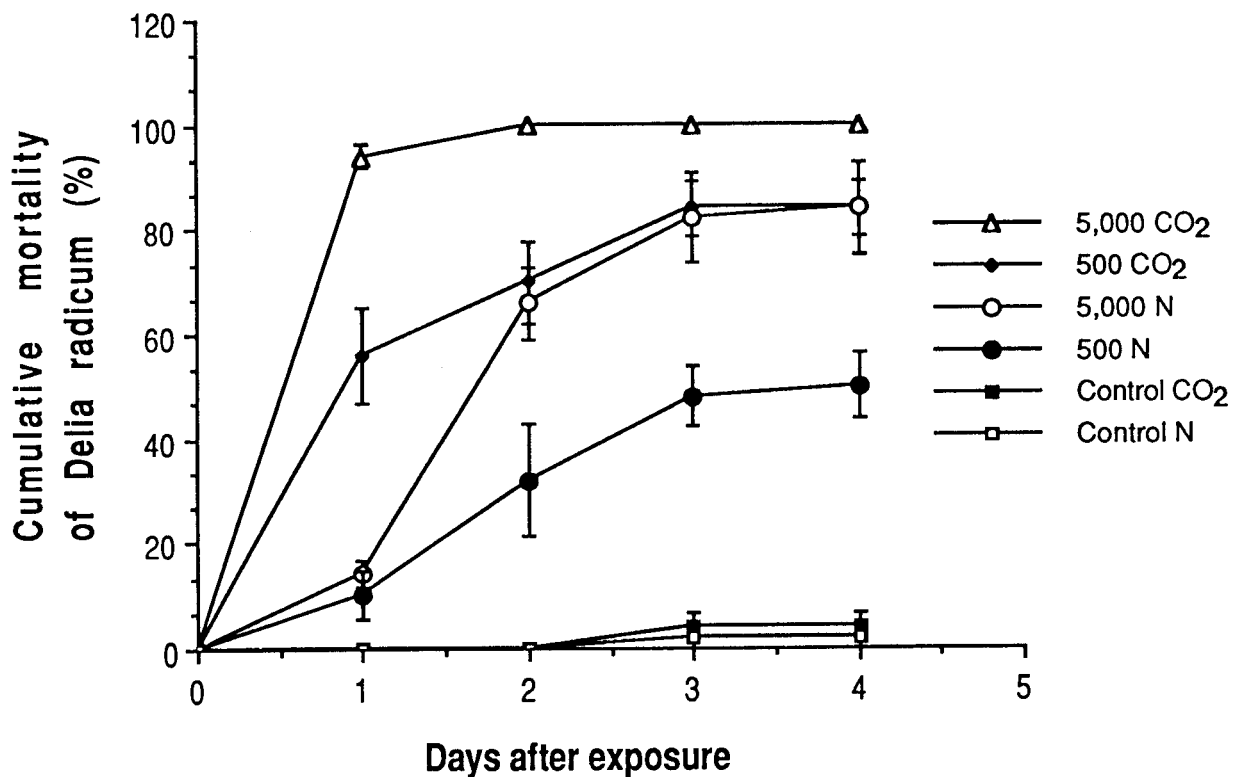


Fig. 17. Mean cumulative mortality of 3rd-instar larval *Delia radicum* (including subsequently-formed puparia), pretreated with CO₂ (CO₂) and normal (N), then exposed to two doses (5,000 and 500) of infective juvenile *Heterorhabditis* strain NC162 for 4 days, at 21.3-22.3 C. Bars stand for standard errors (SE) (n=5).

Table 2. P values* from the comparisons of cumulative mortality of 3rd-instar larval Delia radicum (including subsequently-formed puparia), pretreated with CO₂ (CO₂) and normal (N), then exposed to two doses (5,000 and 500) of infective juvenile Heterorhabditis strain NC162 for 4 days, at 21.3-22.3 C.

	5,000 CO ₂	500 CO ₂	5,000 N	500 N	Control CO ₂	Control N
5,000 CO ₂		P<0.005 P<0.025	P<0.005 P<0.025	P<0.001 P<0.001	P<0.001 P<0.001	
500 CO ₂	P<0.005 P<0.025		P>0.05 P>0.05	P<0.01 P<0.01	P<0.001 P<0.001	
5,000 N	P<0.005 P<0.025	P>0.05 P>0.05		P<0.01 P<0.01		P<0.001 P<0.001
500 N	P<0.001 P<0.001	P<0.01 P<0.01	P<0.01 P<0.01			P<0.005 P<0.001
Control CO ₂	P<0.001 P<0.001	P<0.001 P<0.001				P>0.05 P>0.05
Control N			P<0.001 P<0.001	P<0.005 P<0.001	P>0.05 P>0.05	

* In a row, upper values are from the comparisons within the first 2 days; lower values, within the first 3 days.

4. Puparia

Since the pupal stage of the cabbage maggot does not move in the soil once it has formed, it would be desirable if the nematodes could enter the puparium and infect the pupa within. To clarify this problem, two experiments were conducted.

(1). Using 1-day-old puparia

One-day-old puparia were obtained by the methods described earlier. They were sterilized with 80% alcohol for 1 min. A thin layer of autoclaved sand was added to each of 20 Petri dishes (40x12 mm). Using a pipette, about 2,000 infective juveniles of strain NC162 in aqueous suspension were applied to the sand in each of ten dishes. Surplus water was absorbed with filter paper to keep the sand just moist enough to allow the nematodes to move freely. To each of the other ten control dishes, only distilled water was added to keep the sand about as moist as those in the treated dishes. Then, five puparia were picked up at random with forceps and placed on the sand of each dish, alternately in the treatments and the controls. The dishes were sealed with Parafilm and incubated in the dark at 20-25 C.

Seven days later, all these puparia were removed, washed thoroughly with water, dissected, and checked for internal nematodes.

RESULTS

Soon after the nematodes were introduced, many were seen crawling on the puparia in the treated dishes, and most of them were gathered around the posterior ends of some puparia. Most of the puparia had shown the adult characteristics by the end of

this experiment. No nematodes were found inside the puparia in either the treatments or the controls.

(2). Using mature, 3rd-instar larvae

Mature, 3rd-instar larvae were collected from the sand of rutabaga cultures and washed with distilled water prior to use. Using these larvae and infective juveniles of strain NC162, this experiment was conducted on sand in Petri dishes (40x12 mm) prepared by the same methods described in the previous experiment and under similar conditions. There were five larvae and about 2,000 nematodes in each of ten experimental dishes, but five larvae alone in each of ten control dishes.

Examinations were made every 24 h for 4 days, until the larvae had died or pupated. Dead larvae were removed, washed with water, and 1 or 2 days afterwards, dissected and examined for the presence of nematodes. One week after the last examination, the puparia were removed, washed with water, dissected and examined for the presence of nematodes. Those puparia with adult characteristics were considered to be alive, whereas malformed ones and those without adult characteristics at the end of this test were considered to be dead.

RESULTS

Many nematodes gathered around the posterior ends of some newly-formed puparia. This has also been observed earlier.

Over 4 days, 11 larvae were killed by nematodes in the treatments and the cadavers contained a large number of nematodes; in the controls all the larvae had pupated, two of these died but did not contain any nematodes. In the treatments, four out of 39 puparia were dead, three of which contained

nematodes. Those dead pupae without nematodes, in both the treatments and the controls, were brown or brown-yellow in appearance and rotted.

5. Adults

In this experiment, infective juveniles of strain NC162 were tested for their ability to control adult D. radicum.

Three- to 5-day-old puparia of D. radicum were collected from a rutabaga culture, washed with water, and incubated in a Petri dish with moist filter paper at room temperature.

Thirty Petri dishes (100x15 mm) were divided into two groups of 15. Each dish was given a thin layer of autoclaved sand, kept moist by adding distilled water. Then, using forceps, ten puparia were picked up at random and placed on the sand in each dish. The dishes were sealed with Parafilm and incubated under fluorescent light of 16 h/day at an ambient temperature of 19-27 C. One week later, when it was almost time for the adults to emerge, about 2,000 nematodes of strain NC162 in aqueous suspension were pipetted into each dish of one group, and only distilled water to each dish of the control group. A small amount of food for D. radicum adults was placed on lids of small Petri dishes (40x12 mm), one of which was put on the sand in each dish. One day later, however, the food was withdrawn because it had become so wet that a few adult flies became trapped and died.

Adults began to emerge on the day after the nematodes were introduced. Examination was started on that day and continued every day (except for one day, Appendix 11) for 5 days. Dead adults in the treated group were transferred to clean dishes

with moist filter paper to allow internal nematodes to develop. These adults were dissected 3 or 4 days after they were removed, and the nematodes in their heads, thoraxes, and abdomens, were counted and recorded separately. Dead adults in the control group were not removed, and, at the end of this test, they were all dissected and examined for the possible presence of nematodes.

Unemerged puparia were dissected to check for the presence of any internal nematodes 5 days after this test was ended. The rest were placed on moist filter paper, and were dissected 2 weeks afterwards. Any live juvenile nematodes found inside the puparia were collected and exposed to G. mellonella to determine their infectivity.

Comparisons of adult mortality between the treatment and the control were made by Student's t test.

RESULTS

Adult flies began to emerge on the day after the nematodes were introduced. By the last examination, the same percentage (58.7%) of adult emergence had occurred in both the treatment and the control (Appendix 11). The adults were killed by the nematodes soon after they emerged and some were killed before they were completely free of the puparia. Most of them had been killed by the fourth day. There were significant differences ($P < 0.001$) in accumulated mortalities between the treatment and the control for the first 1-4 days after the nematodes were introduced. However, these differences became insignificant by the fifth or sixth day (Fig. 18).

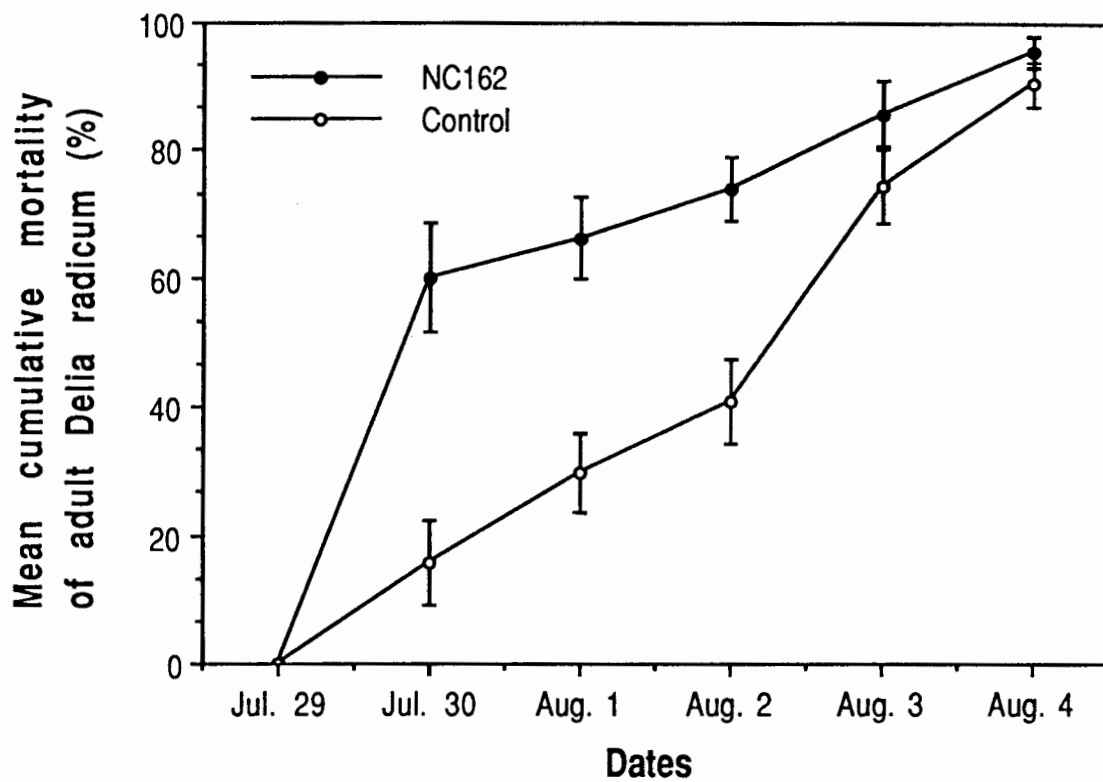


Fig. 18. Mean cumulative mortality of adult *Delia radicum* exposed to infective juvenile *Heterorhabditis* strain NC162 for 6 days, at 19-27 C. Bars stand for standard errors (SE) (n=15).

There were many more nematodes in the abdomens than in the thoraxes ($P < 0.05$) or in the heads ($P < 0.01$), and many more ($P < 0.01$) in the thoraxes than in the heads of the adults (Table 3). In addition, the contents of the intestines of adult nematodes found in the insect heads were red, which was the color of the pigment of the insect's decayed compound eyes.

Of 62 puparia that failed to produce flies by the end of this test, six contained nematodes. Live nematodes recovered from the puparia dissected 5 days after this test was ended were able to kill the larvae of G. mellonella within 3 days. However, those nematodes found in the puparia dissected 2 weeks after this test was ended were all dead.

D. Behavior of Nematodes in the Presence of Host Plants Used by D. radicum

Controlling the cabbage maggot by using nematodes is helped if its host plants do not repel the nematodes. Previous experiments have shown that this insect attracted the nematodes. More nematodes may find the insect if its host plants are also attractive to them. To clarify these problems, the following experiments were conducted to see how nematodes of strain T327, which had been stored in an incubator at 12 C for about 2 months, behaved in the presence of germinated seeds of radish (cv Cherry Belle), and roots of rutabaga (bought commercially), ball cabbage, and radish (cv Cherry Belle). Roots of the last two were grown outdoors from seed.

Table 3. Numbers of infective juvenile Heterorhabditis strain NC162 found in the heads, thoraxes, and abdomens of 57 adult Delia radicum, after 6 days of exposure, at 19-27 C.

	Head	Thorax	Abdomen	Total
No. nemas	177	600	1005	1782
Mean/adult	3.1	10.5	17.6	31.3
SE	0.7	2.0	3.5	5.1

1. In the presence of germinated seeds

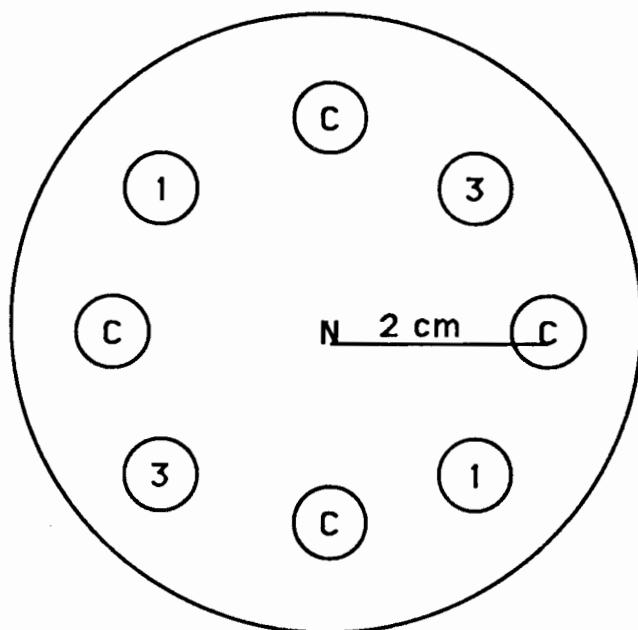
Radish seeds were sterilized with 80% alcohol for 1 min, rinsed five times with sterilized water, and kept overnight on wet filter paper in a Petri dish. They germinated quickly and the radicle grew up to 3 mm long within 24 h at prevailing room temperatures. These germinated seeds complete with testa were transferred to filter paper discs of 7 mm diam which had been arranged on 1.5% Bacto Agar (5 ml/dish) in each of five 100x15 mm Petri dishes as shown (Fig. 19(a)). One seed or three seeds, together with the filter paper discs and the agar underneath, were the treated targets, whereas the paper discs alone and the agar underneath were the control targets. The dishes were sealed with Parafilm and incubated in the dark at 23.2 C for 2 h, so as to allow any chemicals released from the seeds to diffuse into the agar. Then, using a fine hair, about 100-200 infective juveniles of strain T327 were placed at the center of each of the dishes. The dishes were re-sealed and incubated at 24-25 C under fluorescent light. Three hours later, the nematodes on the seeds, on the filter paper discs, and in the agar beneath the paper discs, were counted using the methods described earlier.

Comparisons between the treatments and the control were made by Student's t test, in terms of percentages of nematodes which had reached the targets

RESULTS

Within 3 h, on average, about 1.7%, 3.2%, and 0.5% of the nematodes introduced reached each of 1-seed, 3-seed, and the control targets, respectively (Fig. 20 and Appendix 12). Although most of the nematodes were moving randomly on the agar

(a):



(b):

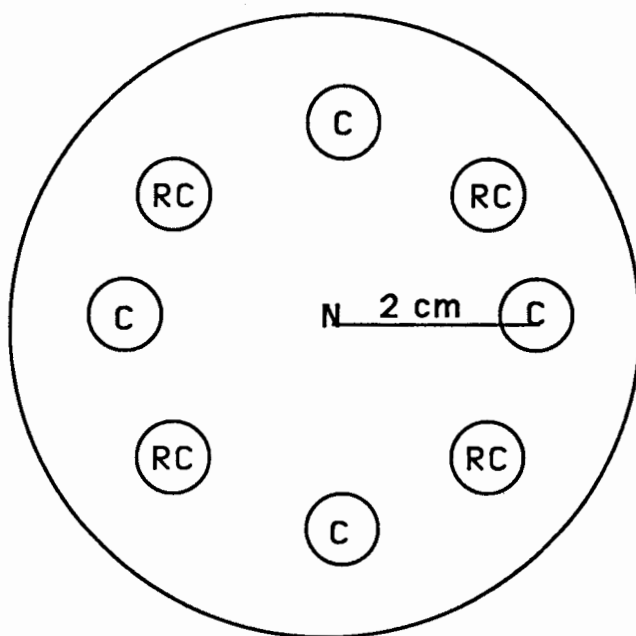


Fig. 19. Arrangement of infective juvenile Heterorhabditis heliothidis strain T327, and (a) germinated seeds of radish; (b) cylinders of radish and rutabaga roots, or of radish and ball cabbage roots, on filter paper discs on agar in Petri dishes. N= nematode introduction point; 1 & 3= number of seeds; RC= root cylinders; C= controls.

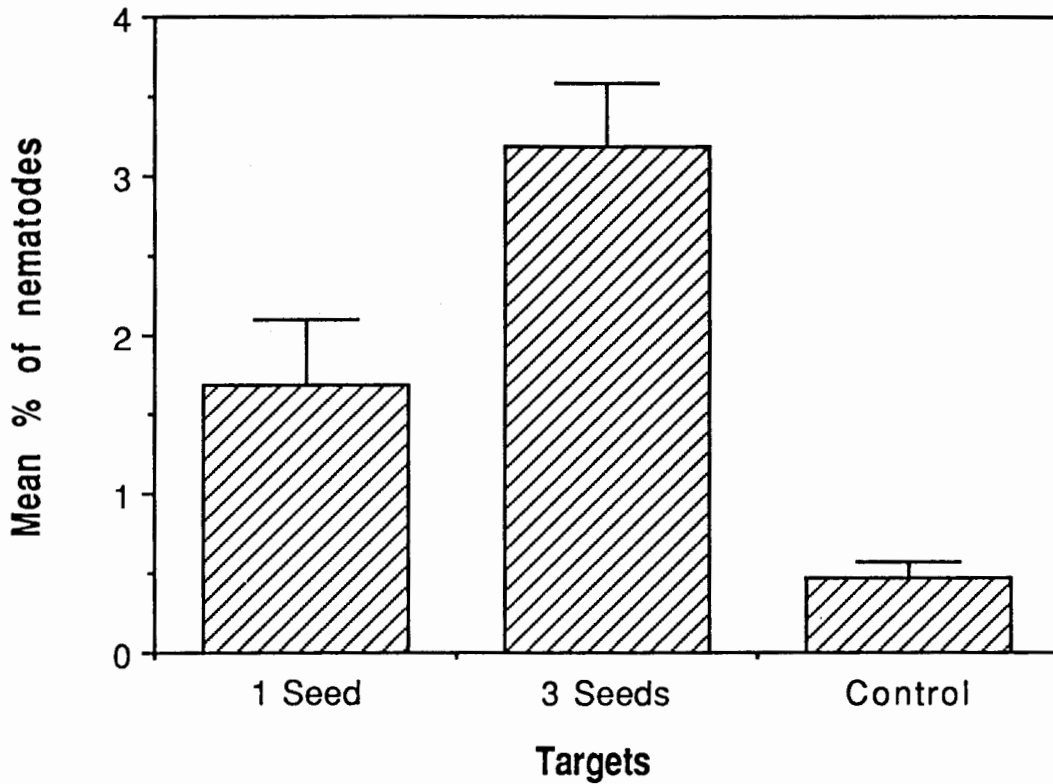


Fig. 20. Mean percent (\pm SE) infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of germinated seeds of radish (ten replicates), and targets of the control (twenty replicates), within 3 h, at 24-25 C.

or had moved to the walls or lids of the dishes, the germinated seeds appeared to be slightly attractive to them, because there were significantly more nematodes at the one-seed ($P < 0.05$) and three-seed ($P < 0.001$) targets than at the controls (Fig. 20). Significantly more nematodes had accumulated at the three-seed than at the one-seed targets ($P < 0.05$) (Fig. 20).

2. In the presence of roots

Two tests, experiment 1 with roots of radish (cv Cherry Belle) and rutabaga, and experiment 2 with roots of the radish and ball cabbage, were run on two different days. During experiments 1 and 2, ambient temperatures were 23.8–24.2 C and 23–24.4 C, respectively. Roots of radish, rutabaga, and ball cabbage, were cut across with a blade and punched with a core punch into cylinders (without root skin) of 5 mm diam and 2 mm height. The cylinders were arranged on filter paper discs of 7 mm diam on 1.5% Bacto Agar (5 ml/dish) in 100x15 mm Petri dishes (Fig. 19(b)). The dishes were sealed with Parafilm and incubated under fluorescent light for 1.5 h. Then, using a fine, mounted hair, about 300–600 nematodes were placed at the center of each dish. The dishes were re-sealed and incubated under light for 2.5 h, at which time the nematodes that had reached the treated and control targets were recorded by the methods described earlier.

Comparisons of attractiveness between the treatments and the controls were made by Student's t test.

RESULTS

Within 2.5 h, on average, in experiment 1, about 13.8%, 0.8%, and 0.5% of the nematodes introduced had reached each of

radish, rutabaga, and the control targets, respectively (Fig. 21(a) and Appendix 13(a)); and, in experiment 2, about 9.4%, 7.2%, and 0.3% of the nematodes had reached each of radish, ball cabbage, and the control targets, respectively (Fig. 21(b) and Appendix 13(b)).

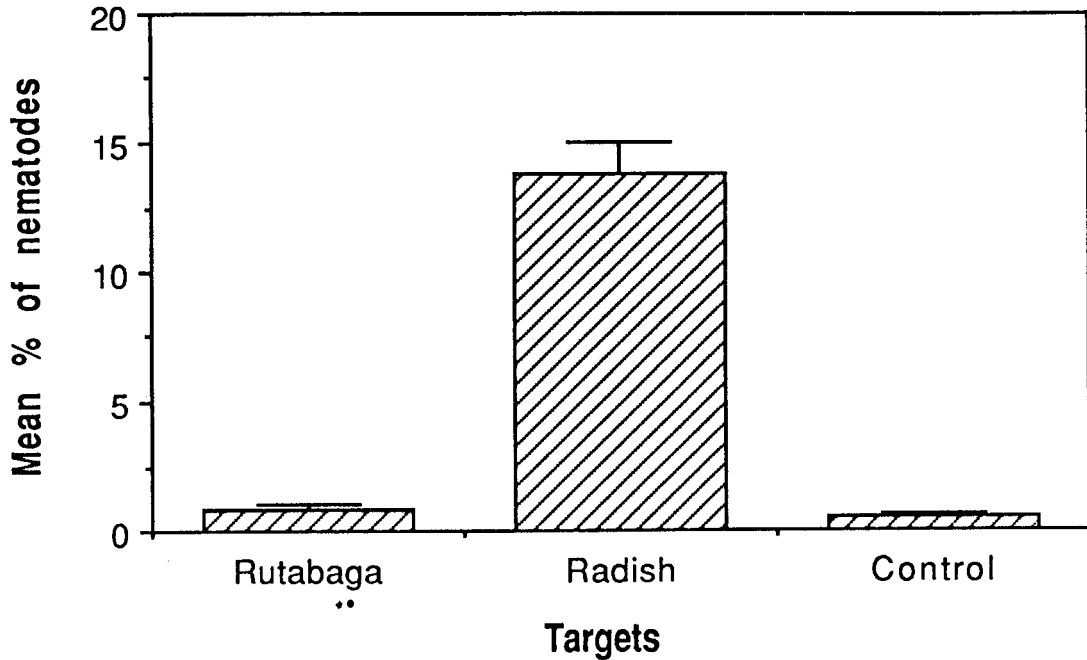
In both experiments, significantly more ($P < 0.001$) nematodes reached radish than the control targets (Fig. 21(a) and Fig. 21(b)), and in experiment 2, significantly more ($P < 0.001$) nematodes were attracted to ball cabbage than to the control targets (Fig. 21(b)). However, there were no significant differences between the attractiveness of rutabaga and the control targets in experiment 1 (Fig. 21(a)), and between radish and ball cabbage targets in experiment 2 (Fig. 21(b)).

E. Field Trial

The object of the field trial was to test the practical use of these nematodes against the cabbage maggot on radish and kohlrabi under field conditions. Since insufficient yield of infective juveniles of strain T327 had been obtained, only infective juveniles of strain NC162 were used.

The trial was conducted during the summer of 1989 on two plots of sandy loam soil, which sloped slightly to the east and were each about 2 m wide and 6 m long, in a garden on the campus of Simon Fraser University. These two plots had been fallow and occupied by various weeds for nearly a year. One of the plots (plot I), had been treated with lime by the previous user. The weeds were lifted by hand, and drainage ditches of about 40 cm wide and 20 cm deep were dug around and between the plots. Prior

(a):



(b):

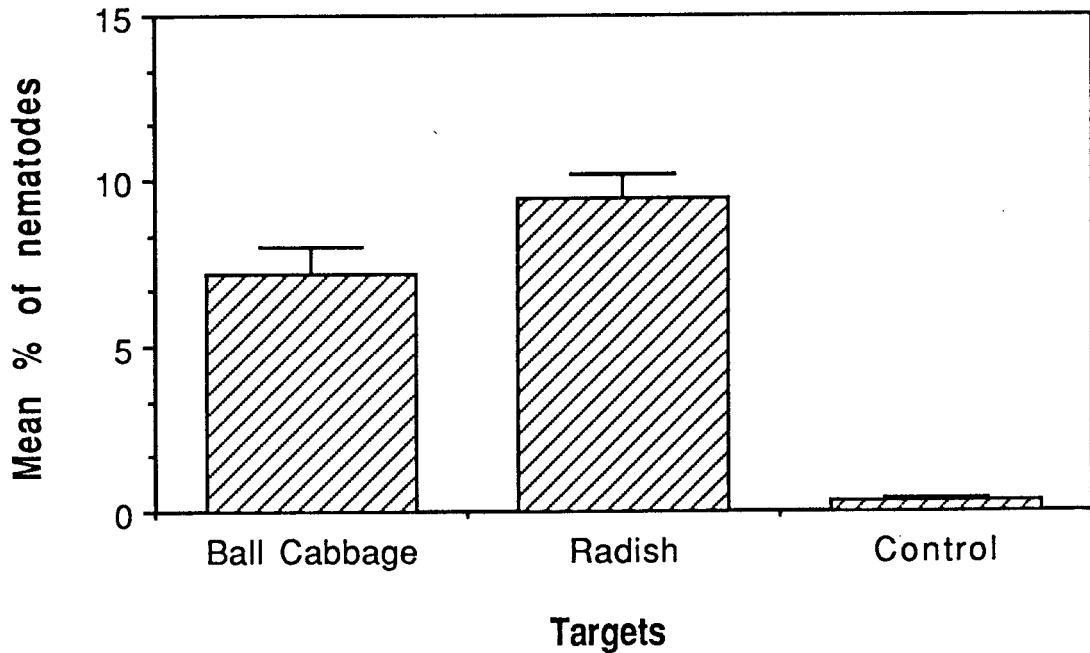


Fig. 21. Mean percent infective juvenile *Heterorhabditis heliothidis* strain T327, which reached targets of cylinders of: (a) radish and rutabaga roots (ten replicates), and targets of the control (twenty replicates), at 23.8-24.2 C; and (b) radish and ball cabbage roots (ten replicates), and targets of the control (twenty replicates), at 23-24.4 C; within 2.5 h. Top bars stand for standard errors (SE).

to being seeded, the soil was mixed evenly with cow manure to a depth of about 5 cm.

Three days later, on May 22, when it rained slightly, seeds of radish (cv Cherry Belle) and kohlrabi were sown manually at 15 cm intervals in holes about 2 cm deep, with three or four seeds per hole. In each plot, seeds of radish and kohlrabi were sown alternately by rows, 20 cm apart, with plot margins of 20-30 cm left unseeded. In each plot, there were 30 rows, 15 each of radish and kohlrabi. Cotyledons of radish emerged within 7 days, and those of kohlrabi within 10 days. From then on, weeds in the crop rows were pulled whenever their populations were considered to be high enough to inhibit crop growth.

On June 15-16, both radishes and kohlrabi were thinned to one plant at each spot. Empty spots where the seeds failed to germinate were filled by transplanting the thinned plants. The radish plants grew quickly, but a small number of them were harmed by slugs. This problem was overcome by applying Meta Slug Killer Bait around the plots at intervals of about 2 m. On June 19, 4 L of fertilizer solution (20-20-20) was applied to the plants in each plot, at 1 teaspoon of 20-20-20 particle/2 L water. Each plant received approximately equal amounts.

One day later, after the plots had been watered with a garden nozzle, three fresh eggs of D. radicum were placed at the base of each radish with a soft brush. The eggs were covered with a thin layer of soil. After three days, when most of the eggs had presumably hatched, the plots were watered for 30 min and then 1.5 h later at 8:30-9:30 pm, about 5,000 infective juveniles of strain NC162 in 0.5 ml aqueous suspension were

applied with a pipette around each radish in rows of treatment I in plot I, and 50,000 such nematodes in 5 ml aqueous suspension around each radish in rows of treatment II in plot II. Radishes in the control rows of both plots did not receive any nematodes. The treatment and control rows alternated, starting from the west sides of the plots. The plots were watered with a fine spray whenever the soil looked dry.

On June 30, two thermometers were buried in the soil at the center of the internal long edge of plot 2, with one at a depth of 5 cm and the other at 10 cm. Minimum and maximum temperatures were recorded by reading at 4:00-6:00 pm daily for 24 days. The thermometers were re-buried after each reading.

Using the same method described for radishes, three fresh eggs of D. radicum were placed around the base of each kohlrabi stem on July 7, and the same doses of NC162 nematodes used with radishes were applied to kohlrabi in the treatment rows on July 10. The treatment and control rows also alternated starting from the west side of each plot. The plots were watered before the eggs were introduced, but not before the nematodes were applied as it had just rained slightly.

On July 11, 3 weeks after the eggs of D. radicum had been introduced, the radishes were dug up with trowels, one by one, together with the soil about 5 cm around them and down to 10 cm deep. The soil and surface of each radish were checked for D. radicum larvae and puparia by spreading the soil on a plastic tray. The radishes from the treatment and control rows were put in four separate, labelled, plastic bags. These fresh radishes, with leaves, were weighed. Then, using a brush, the radishes

were washed with water, and any larvae of D. radicum found in the wash were recorded.

One day later, damage caused by D. radicum on the radishes was assessed, although the work was made somewhat difficult by the damage caused by slugs. Maggot damage was graded as 0, 1, 2, 3, 4, and 5. A damage index was set up by choosing six radishes with typical and distinct damage of these grades. The criteria were as follows: 0--no damage; 1--a radish with 1 tunnel; 2--with 2 or 3 tunnels; 3--with 4 or 5 tunnels; 4--with a big, rotted hole or with more than 5 tunnels; 5--nearly the whole radish rotted or dead. Thus, individual radishes were graded by referring to this index. The radishes were then cut open to locate the internal maggots.

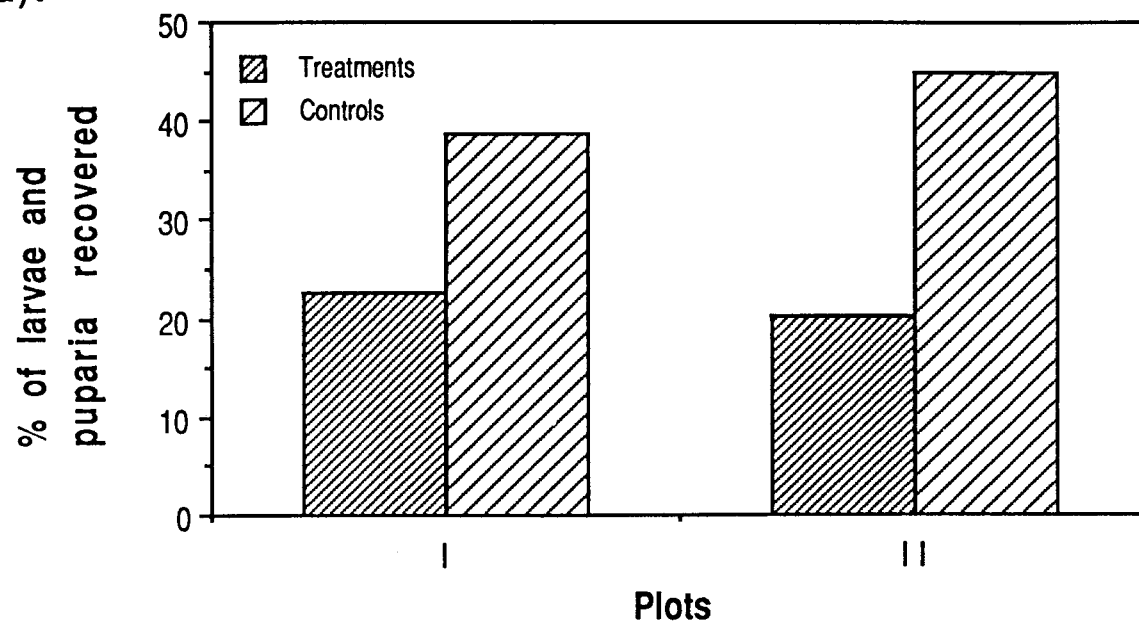
On July 27-29, using the methods described for the radishes, the kohlrabi were harvested, weighed, and washed. Larvae and puparia of D. radicum found in the soil and wash were recorded. Maggot damage on the roots was graded using a 0-5 root damage index of the following criteria: 0--no damage; 1--slight damage on the root surface; 2--1/4 of the root cortex destroyed; 3--1/2 of the root cortex destroyed; 4--3/4 of the root cortex destroyed; 5--total root cortex or nearly the whole root destroyed, or the plant died. The roots were then cut open to find internal larvae.

The efficacy of control by the nematodes of D. radicum on radishes and kohlrabi in this trial was evaluated by making comparisons between the treatments and the controls using the method of Goldstein (1964).

RESULTS

The results of this trial are summarized in Appendixes 14 and 15. With the radishes the results appeared to be positive. As shown in Figs. 22(a) and 23(a), there were significant differences between the treatments and the controls (except for the percentage of radishes with grade 5 damage between treatment II and control II), in the percentages of: a) radishes with no damage ($P < 0.05$ for treatment I and control I; $P < 0.01$ for treatment II and control II); b) radishes with grade 5 damage ($P < 0.05$ for treatment I and control I); c) puparia and larvae recovered from the soil and roots ($P < 0.01$ for treatment I and control I, and for treatment II and control II); d) puparia recovered from the soil ($P < 0.01$ for treatment I and control I, and for treatment II and control II); e) larvae recovered from the soil and roots ($P < 0.05$ for treatment I and control I; $P < 0.01$ for treatment II and control II). In terms of measurements a) to e), there were no significant differences between treatments I and II, and between controls I and II, but between treatment I and treatment II, there were significant differences ($P < 0.01$) in the percentage of puparia recovered from the soil (Appendix 14(a)). In addition, there were five and seven empty pupal cases found in the soil of control I and control II, respectively. Some of the larvae recovered from controls I and II were found infected with nematodes (Appendix 14(a)). These nematodes, as well as those extracted from the larvae recovered from treatments I and II, were able to kill larval G. mellonella within 48 h, and the insect cadavers did not glow in the dark.

(a):



(b):

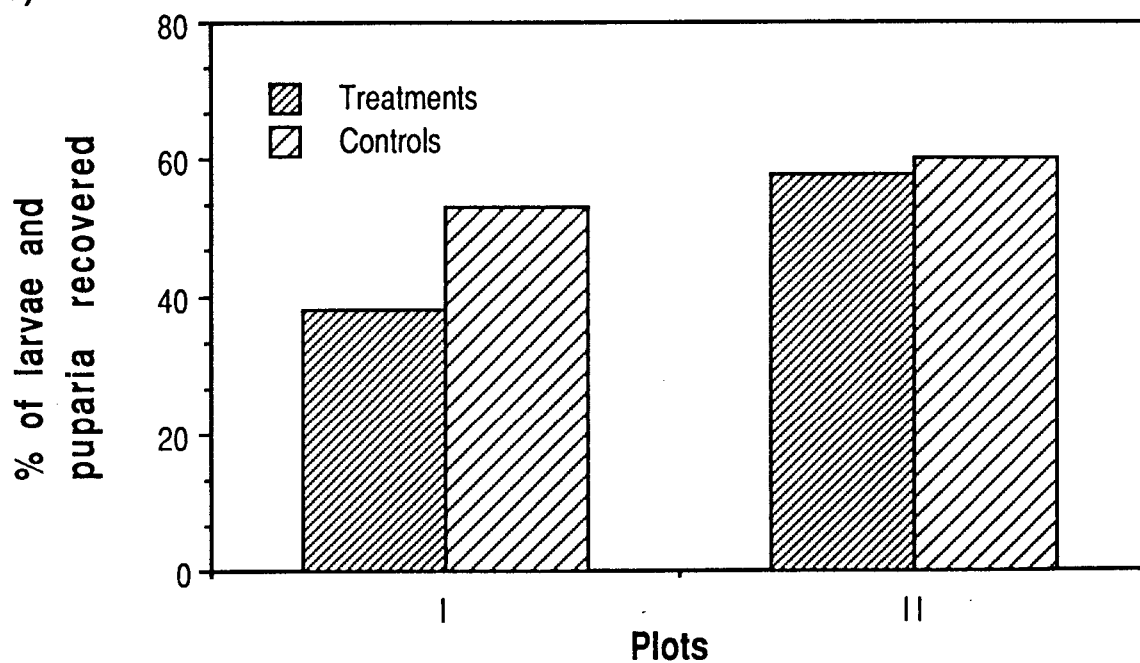
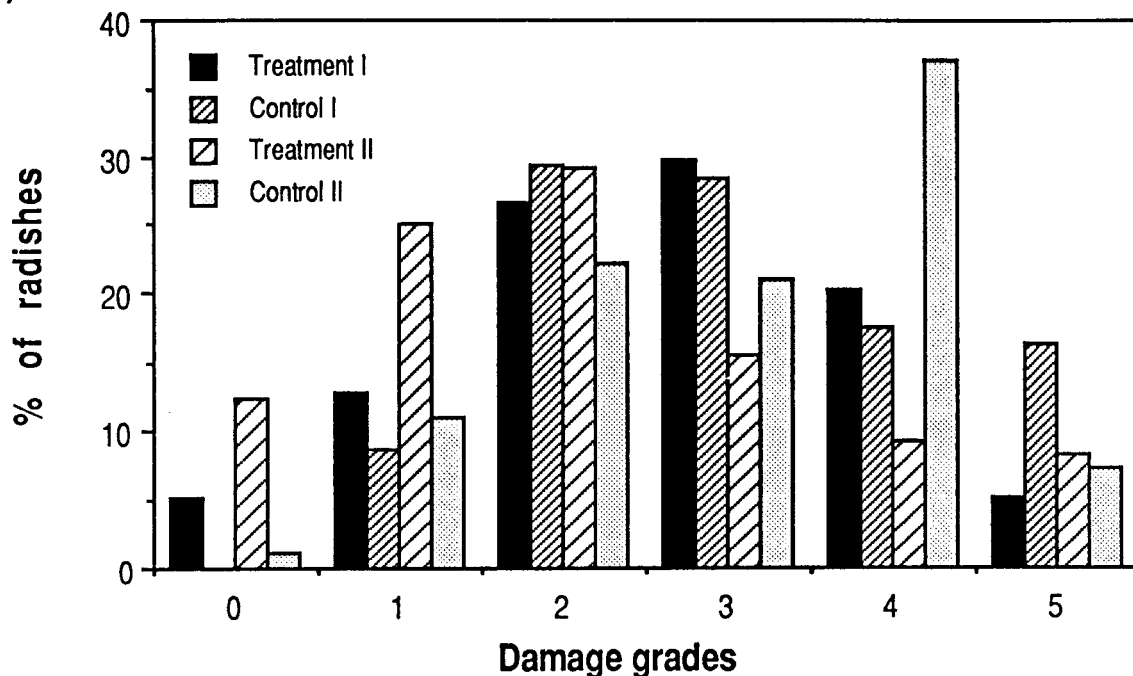


Fig. 22. Percent larvae and puparia of *Delia radicum* recovered from the soil and roots of (a) radishes, and (b) kohlrabi, in the field treated with infective juvenile *Heterorhabditis* strain NC162 (at 5,000 per plant in plot I and 50,000 per plant in plot II), and from controls I and II.

(a):



(b):

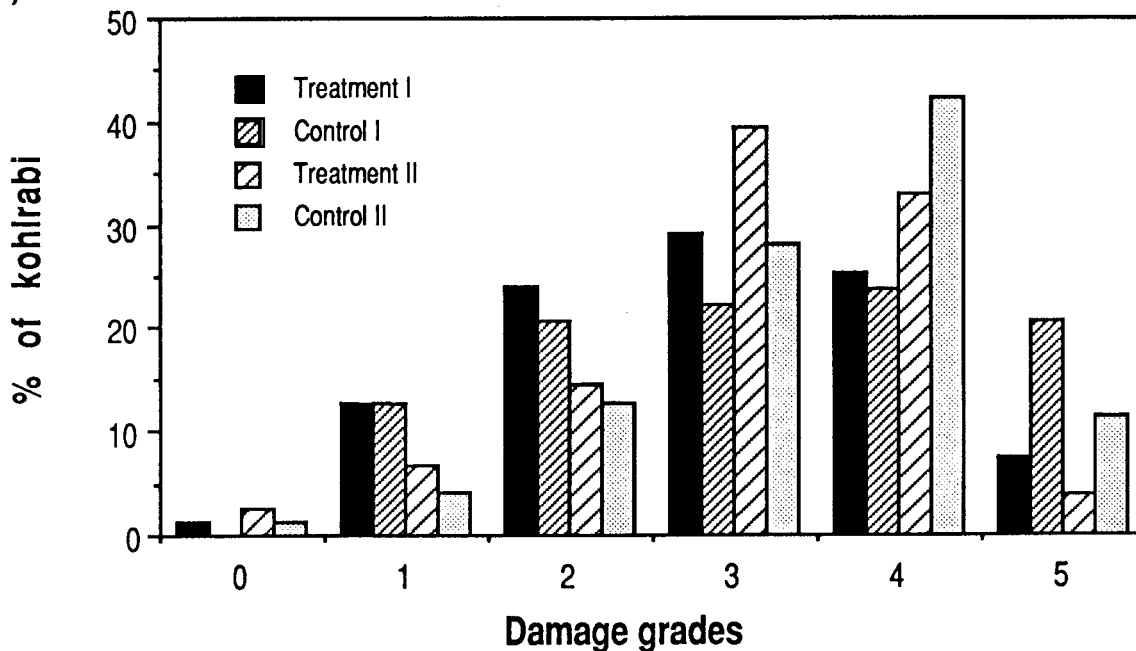


Fig. 23. Percent (a) radishes and (b) kohlrabi roots, with grade-0-5 damage caused by *Delia radicum* in the field treated with infective juvenile *Heterorhabditis* strain NC162, at 5,000 per plant in treatment I and 50,000 per plant in treatment II, and in controls I and II. (Explanation of the "Damage grades" is given in the text).

The results with kohlrabi are reported in Appendix 15 and Figs. 22(b) and 23(b). Fewer roots in treatment I than in control I suffered from grade 5 damage ($P < 0.05$); more larvae and puparia or more puparia alone were recovered from control I than from treatment I ($P < 0.01$); but between treatment I and control I, differences in percentages of roots with no damage and of larvae recovered, were not significant. In terms of the five measurements mentioned in the case of radishes, there were no significant differences between treatment II and control II, except that the differences in percentages of puparia recovered were significant ($P < 0.05$). Between treatments I and II, there were no significant differences in percentages of roots with no damage and with grade 5 damage; but there were significant differences ($P < 0.01$) in percentages of puparia and larvae recovered, and in percentages of puparia alone recovered ($P < 0.05$). Between controls I and II, there were significant differences ($P < 0.05$) in percentages of puparia recovered, but not in the other four measurements. Furthermore, only one larva in treatment I and two larvae in control I were found infected with nematodes (Appendix 15(a)).

There were no significant differences in mean wet weight of whole plants of radish and kohlrabi between the treatments and the controls.

CHAPTER 4: DISCUSSION

A. Selection of Nematode Strains for Controlling the Cabbage Maggot

From the results of this study, it can be concluded that NC162, A13-5 and Breton were lethal to the maggots, although the effect of Breton occurred slightly later (Fig. 3). My observations (unpublished data) in rearing the cabbage maggot showed that on rutabaga, as on other host plants, damage caused by this insect increased rapidly as the maggots grew, especially damage caused by the third-instar larvae. It is thus desirable to kill the maggots as early as possible. As shown in Fig. 3, for the first 5 days after exposure, strain NC162 caused much higher ($P < 0.01$) maggot mortality than did strains DD136 and Breton; A13-5 caused higher ($P < 0.05$) maggot mortality than did DD136 but not higher than Breton. Thus, NC162 appears to have been the best strain against the maggots under the conditions of this experiment. Moreover, my work showed that NC162 could be reared more easily, and because the production was higher than A13-5, NC162 was chosen for later experiments in both the laboratory and the field.

Thurston and Yule (1990) found significant differences between two steinernematid nematode species in virulence against larval northern corn rootworm, Diabrotica barberi, whereas Bedding, et al. (1983) concluded that no one species or strain of Heterorhabditis and Neoplectana (=Steinernema) tested was the most infective for G. mellonella and other insect species. However, insect mortality in the tests of the latter workers was not measured on different days, but over a period of 14 days

instead. This may have resulted in a failure to identify which nematode species or strain would work faster.

Finding that Heterorhabditis strains NC162 and A13-5 are better controls than steinernematids against the maggots agrees with the results of Bedding, et al. (1983) and Molyneux, et al. (1983), for which G. mellonella, sheep blowfly (L. cuprina), and other insects were used as targets. Their success may be partially due to the fact that heterorhabditids possess a terminal tooth-like structure on the head, which is used for directly penetrating the insect cuticle (Bedding and Molyneux, 1982), whereas steinernematids enter a host only through its natural openings, such as mouth, anus, and spiracles (Poinar, 1979). In addition, strain DD136 of S. feltiae (= N. feltiae), a strain tested by numerous workers as virulent against many insect pests, was the least infectious here. This result is similar to that of Bedding, et al. (1983).

Differences in infectivity among some of the nematode strains tested may be less significant when tested with other insects or under different conditions. "Temperature thresholds and other attributes vary for each strain and species and may be more important than relatively small differences in infectivity detected in laboratory tests" (Molyneux, et al., 1983). Strain NC162, considered to be the best candidates against D. radicum in this test, was reared on G. mellonella larvae at 22 C, which is close to natural temperatures in the environment where D. radicum occurs (see under "Field Trial"), and very high nematode production was obtained. Therefore, NC162 was used in later experiments.

Mortalities of tested insects appear to be linearly correlated with nematode dosages (Bedding, et al., 1983). Thus, mortalities of the maggots would be higher than those in this test if higher nematode dosages were applied.

Insect mortality in the nematode treatments were very low for the first 3 days (Fig. 3), because the maggots were inside the rutabaga slices and thereby protected from nematode attack. On the 4th day, having nearly consumed the rutabaga, many of the maggots began to leave the rutabaga and moved onto the filter paper, putting them in closer contact with the nematodes; and at this point, the larger 2nd-instar maggots may have become more attractive to the nematodes. This may account for the rapid increase in mortality of the maggots on the 5th day in the treatments with strains NC162 and A13-5 (Fig. 3).

In the control, there was a rapid increase in mortality of the maggots between the 5th and 6th days (Fig. 3) probably because of starvation. Starvation may also explain why many more maggots died on the 6th day than on the 5th day in the treatments with strains DD136 and Breton (Appendix 1 and Fig. 3), in which most of these newly-dead maggots did not contain nematodes (Appendix 1).

Since D. radicum does its greatest damage in the third instar, the fact that these nematodes kill the maggots in the first and second instars is of interest for bio-control, because the earlier the nematodes take effect against the maggots, the less damage caused.

B. Behavior of the nematodes in the presence of *D. radicum* and its host plants

1. In the presence of *D. radicum*

Results of these studies suggest that the infective juveniles of strains T327 and NC162 responded to living puparia and dead larvae of *D. radicum* by being attracted towards the insects rather than encountering them randomly as they did in the controls. The nematodes apparently reacted to the attractants soon after they were introduced onto the agar, and moved quickly to the targets which they reached within 15 min (Fig. 4).

Since more nematodes reached the targets with time (Figs. 5 and 6), there seemed to be a difference in speed of response to the attractants among individuals of the same strain (T327). Within 4 h, many more nematodes of strain T327 reached targets with dead larvae than targets with living puparia (Appendixes 2 and 3). Both target organisms had been surface sterilized with 80% alcohol, which suggests that the larvae are more attractive than the puparia to the nematodes possibly because they release more attractants. Gaugler, *et al.* (1980) found that the attraction of infective juveniles of *N. carpocapsae* strain DD136 to a CO₂ source was concentration-dependent. The percentage of nematodes aggregating at the source, increased as the flow rate of CO₂ increased from the lower response threshold of <1.0 ml/h to 4.1 ml/h, at which the highest percentage aggregation occurred. Percent aggregation decreased as the flow rate of CO₂ increased from 4.1 ml/h to the upper response threshold of approximately 7 ml/h, at which the nematodes stopped moving and

became straight, probably due to anoxia. They resumed activity when the flow rate was reduced.

Newly-formed puparia of D. radicum are attractive to the one- and two-month-old, infective juveniles of strain T327. Four hours after the nematodes were introduced, there was no tendency for a greater number of puparia to attract more T327 nematodes (Figs. 9 and 11), because the differences among the targets of one, two, and four puparia were not significant. However, such a tendency occurred 24 h after the one-month-old nematodes were introduced (Fig. 11), because there were significant or highly significant differences among the treatments with these three categories of targets ($P < 0.05$ for one and two puparia; and for two and four puparia; $P < 0.005$ for one and four puparia). This fluctuating tendency suggested once more that nematode response to the puparia was weak under the conditions of these experiments.

In the experiments with strains T327 and larvae of D. radicum and G. mellonella, the larvae were attractive to the infective juvenile nematodes. Moreover, in all cases, there was an apparent tendency for multiple larvae to attract more nematodes of both strains (Figs. 12 and 13). This implies that more nematodes may locate a much larger insect host (e.g. four times larger) more easily than a small host. The walls of the dishes seemed to be attractive to the nematodes but it was also possible that the nematodes did not like the agar.

In these experiments, T327 appeared to respond to larval D. radicum more readily than did NC162, because many more T327 nematodes oriented to the targets with four larvae than did

NC162 nematodes ($P < 0.01$). Hence, under certain conditions, better control of insect pests may be achieved by selecting those nematodes which strongly respond to and easily locate their hosts.

In the quantitative experiments, some nematodes of both strains reached the control targets without insects soon after being introduced. This was considered to be accidental because few of these nematodes stayed at the control targets after 4 or 24 h. In these cases, the attractiveness of the insect targets may have lured most of these nematodes away from the control targets.

Some investigators have suggested that certain insect-parasitic nematode species are attracted to their insect hosts by physical or chemical cues. Infective juveniles of strain DD136 were shown to orient to a CO_2 source (Gaugler, et al., 1980). Schmidt and All (1978) found that these juveniles were attracted to various insect larvae from diverse ecological habitats, and to dilutions of an aqueous surface wash of larvae of G. mellonella. They suggest that the attraction is to a chemical gradient around the insect larvae. Common excretory products of insects such as uric acid, xanthine, allantoin, ammonia, and arginine were also attractive to S. feltiae (Schmidt and All, 1979). These nematodes showed concentration-dependent accumulations at gradients of some salts with various cations or anions, and certain pH's, and all gram-negative bacteria tested also caused significant nematode accumulations (Pye and Burman, 1981). Byers and Poinar (1982), however, considered that these nematodes aggregated in response to insect

produced heat (about 0.3 C above ambient) conducting from electric heat sources or from a single larva of G. mellonella, both in the absence of CO₂ and other chemicals.

In the present studies, any or all these factors may have been responsible for attracting the nematodes. Orientation of T327 and NC162 nematodes to D. radicum and G. mellonella may be caused by the combined effect of the factors, some of which may predominate. Microbes on the surface of larval and pupal D. radicum tested in the qualitative experiments had probably been eliminated by sterilization with alcohol; certainly no visible contamination was observed during the whole experimental process. In the quantitative experiments, 26 h after the insects were introduced, there was slight visible bacterial contamination on the agar around some of the filter paper discs with unsterilized insects, but not on the agar around the filter paper discs with sterilized insects. Moreover, unsterilized and sterilized insects were equally and strongly attractive to the nematodes. Therefore, microbes on the surface of these insects appeared to play no part in attracting the nematodes in the tests. Heat, produced from respiration of living pupae or from tissue decomposition of dead larvae due to possible interior microbial activity, might play some part in attracting the nematodes. But this would not be significant because it was unlikely that any one of these small insects would produce as much heat as a much larger, last-instar larval G. mellonella which had a surface temperature about 0.3 C above the ambient (Byers and Poinar, 1982). It was more likely that chemical gradients developed around these insects, such as CO₂ produced

from respiration, and ions or other chemicals released from dead larvae due to possible tissue decomposition caused by interior microbial activity, were mainly responsible for attracting the nematodes. There were no significant differences in attraction between dead larvae of D. radicum and those of G. mellonella in percentages of nematodes which reached targets of larvae of these two species (Fig. 13). This suggests that different insect species may contain similar chemicals and breakdown products that are attractive to the nematodes.

Poinar and Leutenegger (1968) found that amphids and somatic muscles in infective juvenile S. feltiae were much more developed than those in normal third-stage juveniles. These amphids may work as chemoreceptors to react to chemicals released from insects. Highly developed somatic muscles may enhance the ability of the infective juveniles to reach an insect host. Baldwin and Hirschmann (1975) demonstrated that certain papillae, which occur in many surface regions of nematode body, are likely to be chemoreceptive.

Infective juveniles of strain T327 which were collected directly from their rearing cultures did not orient to pupal D. radicum whereas others from the same cultures which had been stored at 12 C for one month did so (Fig. 11). Infective juveniles of strain T327 may, therefore, need a certain lapse of time before they are able to recognize physical or chemical attractants from their insect hosts. It may be necessary that newly-harvested infective juveniles of strain T327 be stored for a certain period before they are used for the control of insect pests. Infective juveniles of strain NC162 collected directly

from their rearing cultures were attracted to pupal D. radicum (Fig. 10). This fact may indicate a difference in development rates between these two strains.

In nature, cabbage maggots are often confined to a single host plant and feed inside the roots. Thus, chemicals released from the maggots could establish a gradient. Moreover, their feeding would release ions and cause bacterial growth. These circumstances may aid the nematodes in locating an insect host.

Results of the experiments described in Chapter 3 indicate that very low percentages of the applied nematodes entered D. radicum. For example, only 1,782 out of 30,000 (=5.94%) infective juveniles of strain NC162 entered 57 adults of this insect. Therefore, the use of these nematodes for the control of this pest would be more economic if those nematodes which are attracted to this insect were selected and bred in quantity.

In addition, the fact herein that infective juveniles of strain T327 were able to enter dead larval D. radicum and G. mellonella and to develop inside is in agreement with the report of Hara and Kaya (1983), who found that S. feltiae invaded and reproduced in insecticide-killed larval Spodoptera exigua (Hubner).

2. In the presence of host plants used by D. radicum

Results of the experiments in section D of Chapter 3 indicate that germinated seeds and root tissue of radish and ball cabbage are all attractive to infective juveniles of strain T327, and that there is an apparent tendency for the greater number of seeds to attract more nematodes. But root tissue of rutabaga neither attracted nor repelled the nematodes. Thus,

using these nematodes to control D. radicum on these host plants appears to be feasible.

As with larval and pupal D. radicum, T327 orients to germinated seeds of radish and to roots of radish and ball cabbage, probably by responding to heat and CO₂ released from the plants as by-products of respiration. What must be mentioned is that cell substances such as various ions, carbohydrates, lipids, proteins, and other chemicals, liberated from the root cylinders by injury, might also be responsible for attracting the nematodes. It seems unlikely that bacterial gradients could have developed around root cylinders and the seeds during a mere 4 or 5 h, because the seeds had been sterilized with 80% alcohol and only the inner parts of the roots were used. Hence, it is probable that no bacterial gradient contributed to the attraction of the seeds and roots.

The fact that the roots of radish and ball cabbage are attractive to the nematodes but those of rutabaga are not may reflect the different chemical composition of these roots.

In nature, plant roots liberate various chemicals such as ions, CO₂, and amino acids (Borner, 1960), which form a chemical gradient around the root system. Heat given off as a by-product of respiration by the roots and produced by rhizospherical microorganisms may establish a temperature gradient around the root system. These chemical and temperature gradients may attract the nematodes and in turn increase the opportunity for the nematodes to obtain access to larval D. radicum which feeds on or inside the roots of its host plants. This may explain why

some of the larvae found inside the radishes in the field trial contained nematodes.

In conclusion, the combined attractiveness of D. radicum and its host plants to these nematodes may aid the nematodes in locating their insect hosts. Thus, the control of insect pests by using nematodes could be enhanced.

C. Using Selected Nematode Strains to Control the Cabbage Maggot in the Laboratory

Eggs of D. radicum had been exposed to infective juveniles of strain NC162 for 20 h, but the newly-hatched larvae and unhatched eggs did not contain any nematodes. If there were any openings in the shell of the egg, through which the nematodes could enter, 20 h would certainly be sufficient for the nematodes to do so, because the results of later experiments showed that the nematodes were able to enter and kill larval D. radicum within 24 h. Thus, the shell of the egg is apparently a protective barrier to attack by the nematodes.

Newly-hatched maggots placed on sand about 1 cm from or directly on radishes were able to tunnel into the radishes within less than 6 h, during which only 11 out of 200 newly-hatched maggots were killed by the nematodes of strain NC162 (see under "2. First-instar larvae" in Chapter 3, pp. 46-49). Within up to 24 h and 48 h, only 18% and 71% of the maggots were infected with these nematodes, respectively (Fig. 14). From these results it is concluded that strain NC162 does not appear to attack newly-hatched maggots effectively before they enter the host plants. It may take the maggots a longer time to tunnel

into harder roots, such as those of rutabaga, than to tunnel into radishes. This may explain why treatments with nematodes have resulted in a reduction in the degree of damage on rutabaga (Welch and Briand, 1961b).

As shown in Fig. 15, many more ($P < 0.01$) 3rd-instar maggots were attacked by NC162 than by T327. Maggots killed by NC162 were full of nematodes of various ages, whereas those killed by T327 contained many fewer and most of these nematodes failed to develop and died, suggesting that NC162 developed and reproduced better than T327 inside the maggots under the conditions of this experiment. Therefore, strain NC162 appears to be more effective than strain T327 against the maggots.

Eight hours of CO_2 treatment did not have a detectable harmful effect on third-instar larval D. radicum, but rather pacified them for less than 1 h, during which many nematodes were crawling on the larvae, probably searching for a portal of entry. This short period has been proved by the results of this test to be long enough to allow the nematodes of strain NC162 to attack the maggots effectively. There was a tendency for a higher dose of these nematodes to kill more maggots (Fig. 17). But 500 nematodes yielded nearly the same control of the CO_2 -treated maggots as 5,000 nematodes did on normal maggots within 3 days; moreover, the 500 took effect faster than did the 5,000--shown by a highly significant difference ($P < 0.001$) in mortality of the maggots, within the first day (Fig. 17). In addition, the quick, fatal effect of a dose of 5,000 nematodes on the maggots is obvious (Fig. 17). These results suggest that if a practical substance is available, specially able to pacify this pest but

not harmful to the nematodes, humans, plants, or the environment, the control of this pest by nematodes should be improved.

The puparium of D. radicum is a completely closed case formed from the integument of the last-instar larva. Seen under a high-powered microscope, it appears impenetrable to the nematodes. This morphological barrier was apparently able to protect the contained pupa from attack by the nematodes of strain NC162, because no tested one-day-old puparium contained any nematodes even though it had been exposed to them for 7 days. When mature, 3rd-instar larvae were exposed to the nematodes for more than 7 days, as few as three out of 39 later-formed puparia contained nematodes (see under "4. Puparia" in Chapter 3, p. 54). These nematodes had probably entered the larva and were trapped in the puparium which formed subsequently. Dead pupae not containing nematodes were found in both the treatments and the controls. They were brown or brownish-yellow and putrefied; they may have been infected by bacteria from the insect culture. In addition, many nematodes were seen gathering around the tails of some newly-formed puparia. They were probably attracted by physical cues such as heat, or by chemical cues such as CO₂, released from the puparia.

Adult D. radicum are susceptible to attack by the nematodes. As shown in Appendix 11, most adults were killed by the nematodes soon after they emerged, suggesting that their untanned cuticle and low mobility made them more vulnerable to attack as compared with the older flies. This may be significant

for the nematodes as bio-control agents against newly-emerged, adult D. radicum.

Less fly mortality was caused by the nematodes as time elapsed (Fig. 18), suggesting that the nematodes did not persist long in the sand. This is due mostly to the effect of desiccation as it was found on Aug. 1 that the sand in some dishes was dried out. Therefore, application of nematodes in the field must be under moist conditions, and application should be repeated if necessary. The high mortality of the adult flies that occurred in the controls (Fig. 18 and Appendix 11) at the end of this experiment, probably resulted from starvation.

The contents of the intestines of adult nematodes suggest that the nematodes fed on host materials. The abdomens of the flies appear to be more suitable for nematode growth and development than the heads and thoraxes.

The nematodes found in six of the 62 puparia that failed to produce flies by the end of this test entered the puparia probably through injuries caused by handling.

D. Field Trial

In the radishes, there were many more larvae and puparia of the pest ($P < 0.01$) found in the controls than in the treatments with NC162 nematodes (Figs. 22(a)). This suggests that the nematodes could indeed lower a population of D. radicum in the field, even though some damage had occurred in most of the radishes (Appendix 14(b)), which were thus unsalable. But from a long-term point of view, this result may point to a means of preventing the fly population from building up in a field.

In the total percentages of radishes damaged by this pest, there were significant differences between treatment with 5,000 nematodes/plant and the control ($P < 0.05$), and between treatment with 50,000 nematodes/plant and the control ($P < 0.01$). These results showed that the nematodes were able indirectly to reduce the number of damaged radishes probably by killing some of the newly-hatched larvae before they entered the radishes. Significantly ($P < 0.05$) fewer radishes in the treatment with 5,000 nematodes/plant than in the control suffered from grade 5 damage and significantly ($P < 0.01$) fewer radishes in the treatment with 50,000 nematodes/plant than in the control suffered from grade 4 damage (Fig. 23(a)). There were no significant differences between both treatments and between their controls in the percentages of radishes with moderate damage (grades 2 and 3). It appears that once the maggots had entered a plant the nematodes needed some time to gain access to them and kill them at a later stage, so preventing more severe damage.

Insect-parasitic nematodes were attracted to basic pH (Pye and Burman, 1981). Infectivity of the nematodes to the maggots may have been enhanced in the basic environment in treatment I of limed plot I. This may explain why, between treatments I and II, where each radish was respectively treated with 5,000 and 50,000 nematodes, there were no significant differences in the percentages of recovered larvae and puparia from the eggs introduced (Fig. 22(a)), or of total damaged radishes (Appendix 14(b)).

My observations showed that at 23-25 C, D. radicum completed its egg and larval stages within about three weeks, and its pupal stage within at least one week. During the warm period (June 30-July 26, 1989) of this trial, mean daily soil temperatures ranged from 14.54 (± 0.30) C to 27.10 (± 0.65) C, and by the time of harvesting, it had been only 3 weeks since the eggs of D. radicum were introduced. Therefore, it was unlikely that adult flies had emerged. Those empty puparia found in the soil of the controls probably resulted from the contained pupae having been preyed upon by unknown predators.

Digging up radishes, as well as kohlrabi, together with the soil from about 5 cm around the plants and down to 10 cm deep, should have recovered most of the cabbage maggot population. Since the soil of both plots remained moist, most of the mature larvae would pupate within this volume of soil. The fact that the total number of larvae or puparia recovered from each plant and the soil around it did not exceed the three eggs originally introduced per plant, suggested that there was no natural population of D. radicum.

Some factors may account for the presence of nematodes in some of the larval D. radicum recovered from the radishes in the controls. Under laboratory conditions, infective juveniles of H. heliothidis are able to migrate horizontally up to 30 cm in sandy soil within one month in the absence of insects (Schroeder and Beavers, 1987), and infective juveniles of H. bacteriophora Poinar migrated horizontally and killed larval G. mellonella placed 40 cm apart in soil within less than a week (Sandner, 1986). Here in each plot, every two rows of radishes were 40 cm

apart and with one row of kohlrabi in between, but the kohlrabi did not receive eggs of D. radicum until 17 days after the radishes. There were 3 weeks between the dates of egg introduction and radish harvesting. Thus, the nematodes introduced into the treatments of radishes could have migrated to the controls during this period. The attractiveness of D. radicum and radishes for the nematodes probably contributed to the nematode migration. However, rain and irrigation water may have played a more important part in dispersing the nematodes, because both plots sloped slightly from the west to the east side. However, it was also possible that the nematodes in the controls were from an original soil infestation because both plots had been fallow and filled with various weeds for nearly a year, during which some insect-parasitic nematodes could be maintained by a natural source of insect hosts. In fact, my work showed that the nematodes extracted from the larvae in both the treatments and the controls were able to kill larval G. mellonella within 48 h and the insect cadavers did not glow in the dark; and that the 1st generation adults of these nematodes were hermaphroditic. All these nematodes appeared to be those originally applied. Hence, I consider it more likely that the nematodes found in the controls of this trial had moved from the treatments.

There were no significant differences in the percentages of larvae infected with nematodes between the treatments and the controls (Appendix 14(a)). This may have resulted because the insect cadavers infected earlier in the treatments might have decayed by the time of harvesting, whereas those in the controls

were probably freshly infected, for the nematodes needed a certain period to migrate or to be dispersed from the treatments to the controls.

The results with kohlrabi were inconclusive. Significantly ($P < 0.01$) fewer larvae and puparia were recovered from treatment I than from its control (Fig. 22(b)), but differences between the two in the percentages of damaged plants and of plants with individual-grade (except grade 5) damage, were not significant (Fig. 23(b) and Appendix 15(b)). The results suggest that the nematodes may have killed more insects in treatment I than in its control, but this reduction of the population was probably not great enough that the survival in treatment I caused nearly as much damage as that in its control. The nematodes found in control I could have migrated or been dispersed from the treatments by means similar to those in the test with radishes.

The results with kohlrabi from both plots suggest that the nematodes of strain NC162 did not have any significant effect on the population of D. radicum, nor was the damage reduced. This may be explained as follows. Most of the infective juveniles of H. heliothidis remained at the 0-2 cm depth (Georgis and Poinar, 1983c), 5 days after they were placed on the soil surface, even though they were in the presence of G. mellonella placed underneath. Moreover, UV radiation and natural sunlight have been shown to be fatal to insect-parasitic nematodes (Gaugler and Boush, 1978; Gaugler, et al., 1989). Thus, many of the nematodes applied on the soil surface in this trial might have been killed by sunlight and desiccation. This adverse effect on the nematodes was probably worse with kohlrabi than with

radishes, because the soil was more compact during the period when the kohlrabi were tested, making it difficult for the nematodes to move downwards. This situation may also explain why so few larvae were infected with nematodes in the treatments.

CHAPTER 5: CONCLUSIONS

Differences in infectivity exist among entomopathogenic nematode species/strains. *H. heliothidis* strain NC162 appeared to be the most virulent against larval *D. radicum* in this study. The egg shells and puparia of *D. radicum* are able to protect the eggs and pupae from the attack by these nematodes. Larval and adult *D. radicum* are susceptible to the nematodes. Therefore, control of the pest by the nematodes should be focussed on the latter two stages. To prevent root damage, newly-hatched maggots must be killed before they enter their host plants. The nematodes seemed to be ineffective in carrying out this mission, probably due to the active movement of the maggots and their limited openings through which the nematodes can enter. However, if a practical substance is available, able to pacify the maggots but not harmful to other organisms or the environment, the control of this pest should be improved. In the present study this hypothesis was tested by treating 3rd-instar maggots with CO₂ before applying nematodes. The fact that untanned adult *D. radicum* were especially vulnerable to nematode attack is of interest and would make controlling the pest possible if the timing of nematode application is carefully considered. The potential of using nematodes as biocontrol agents against *D. radicum* has been further supported by the results of the field trial, wherein strain NC162 lowered the population of this pest and reduced its damage in radishes. Moreover, the combined attractiveness of *D. radicum* and its host plants to these nematodes may aid the nematodes in locating their insect hosts.

Thus, the control of this pest by using nematodes could be enhanced.

REFERENCES CITED1. On the Cabbage Maggot

- Abu Yaman, I.K. 1960. Natural control on cabbage root fly populations and influence of chemicals. Meded. Landb Hoogeschool. Wageningen. 60: 1-57.
- Beirne, B.P.. 1971. Hylemya brassicae (Bouché), the cabbage maggot. In Pest Insects of Annual Crop Plants in Canada. I. Lepidoptera. II. Diptera. III. Coleoptera. Memoirs Entomol. Soc. Can. No.78: 53-56.
- Bonnemaison, L. 1965. Insect pests of crucifers and their control A. Rev. Ent. 10: 233-256.
- Brittain, W.H. 1927. The cabbage maggot (H. brassicae Bouché) and its control in Nova Scotia. Bull. Nova Scotia Dep. nat. Resour. No. 11.
- Brooks, A.R. 1951. Identification of the root maggot (Diptera: Anthomyiidae) attacking cruciferous garden crops in Canada, with notes on biology and control. Can. Ent. 83(5): 109-123.
- Coaker, T.H. 1965. Further experiments on the effect of beetle predators on the numbers of the cabbage root fly, Erioischia brassicae (Bouché), attacking brassica crops. Ann. Appl. Biol. 56: 7-20.
- Coaker, T.H. 1966. The effect of insecticides on the predators and parasites of the cabbage root fly (Erioischia brassicae (Bouché)) and on subsequent damage caused by the pest. Ann. Appl. Biol. 57: 349-354.
- Coaker, T.H. 1967. Insecticidal control of cabbage root fly (Erioischia brassicae (Bouché)) in the axillary buds of Brussels sprouts. Ann. Appl Biol. 59: 339-347.
- Coaker, T.H., D.J. Mowat and G.A. Wheatley. 1963. Insecticide resistance in the cabbage root fly in Britain. Nature. Lond. 200: 664-665.
- Coaker, T.H. and S. Finch. 1965. The control of cabbage root fly on edible brassicae. Pl. Path. 14: 1.
- Coaker, T.H. and S. Finch. 1967. The behaviour of the cabbage root fly. Rep. Natn. Veg. Res. Stn. for 1966. 61-62.
- Coaker, T.H. and S. Finch. 1971. The cabbage root fly, Erioischia brassicae (Bouché). Report of National Vegetable Research Station for 1970. pp. 23-42. Wellesbourne, Warwick.

- Dimond, J.B., O.L. Arden. and D.M. DeLong. 1956. Nutritional requirements for reproduction in insects. Int. Congr. Ent. 2: 135-138.
- Doane, J.F. and R.K. Chapman. 1964. The relation of the cabbage maggot, Hylemyia brassicae (Bouché) to decay in some cruciferous crops. Ent. exp. & appl. 7: 1-8.
- Finch, S. 1971. The fecundity of the cabbage root fly Erioschia brassicae under field conditions. Ent. exp. & appl. 14: 147-160.
- Friend, W.G. 1956. The nutrition of phytophagous insects with special reference to Hylemya antiqua (Mg.). Int. Congr. Ent. 2: 145-150.
- Gibson, A. and R.C. Treherne. 1916. The cabbage root maggot and its control in Canada. Bull. Can. Dept. Agr. Ent. No.12.
- Harris, C.R., G.F. Manson and J.A. Mazurek. 1962. Development of insecticidal resistance by soil imsect in Canada. J. Econ. Ent. 55: 777-780.
- Harris, C.R. and H.J. Svec. 1966. Mass rearing of the cabbage maggot under controlled environmental conditions, with observations on the biology of cyclodiene-susceptible and resistant strains. J. Econ. Ent. 59(3): 569-573.
- Hassan, S.A. 1969. Observations on the effect of insecticides on Coleopterous predators of Erioischia brassicae. (Dipt., Anthomyiidae). Ent. exp. and appl. 12: 157-168.
- Hawkes, C. 1968. The behaviour of the adult cabbage root fly. Field behaviour. Rep. natn. Veg. Res. Stn for 1967. p. 66.
- House, H.L. 1954. Nutritional studies with Pseudosarcophaga affinis (Fall.), a dipterous parasite of the spruce budworm, Choristoneura fumiferana (Clem.). II. Effects of eleven vitamins on growth. Can. J. Zool. 32: 342-350.
- Howitt, A.S. and S.G. Cole. 1962. Chemical control of Hylemya brassicae (Bouché) in the Pacific Northwest. J. Econ. Ent. 55: 33-38.
- Hughes, R.D. 1959. The natural mortality of Erioischia brassicae (Bouché) (Diptera, Anthomyiidae) during the egg stage of the first generation. J. Anim. Ecol. 28: 343-357.
- Hughes, R.D. 1960 Induction of diapause in Erioischia brassicae Bouché (Dipt., Anthomyiidae). J. Exp. Biol. 37: 218-223.
- Hughes, R.D. and B. Mitchell. 1960. The natural mortality of Erioischia brassicae (Bouché) (Diptera, Anthomyiidae): life tables and their interpretation. J. Anim. Ecol.. 29: 359-374.

- Hughes, R.D. and D.D. Salter. 1959. Natural mortality of Erioischia brassicae (Bouché) (Diptera, Anthomyiidae) during the immature stages of the first generation. J. Anim. Ecol.. 28: 231-241.
- Lowry, Q.S. 1914. The cabbage root maggot. Rep. Conn. Agric. Exp. Stn for 1914. pp. 142-152.
- Mackenzie, J.R., R.S. Vernon and S.Y. Szeto. 1987. Efficacy and residues of chlorpyrifos applied against root maggots attacking cole crops in British Columbia. J. Entomol Soc. Brit. Columbia. 84: 9-16.
- MacLennan, A.H. 1922. New methods developed in control of insects and fungus diseases. Rep. Ont. Veg. Growers Assoc. for 1921: 32-38.
- Matthewman, W.G. and L.H. Lyall. 1966. Resistance in cabbage to cabbage maggot, Hylemya brassicae (Bouché). Can. Ent. 98: 59-69.
- Miles, M. 1952. Studies of British Anthomyiid flies. Bull. Entomol. Res. 43: 83-90.
- Miles, H.W. The control of the cabbage root fly. J. Minist. Agric. Fish. 37: 1227-1231.
- Morris, R.F. 1960. Control of root maggots in swede turnips in Newfoundland with heptachlor and aldrin and the effect on parasites and overwintering pupae. J. Econ. Ent. 53: 65-67.
- Mowat, D.J. and T.H. Coaker. 1967. The toxicity of some soil insecticides to carabid predators of the cabbage root fly (Erioischia brassicae (Bouché)). Ann. Appl. Biol. 59: 349-354.
- Muesebeck, C.F.W. 1942. Common names of insects approved by the American Association of Economic Entomologists. J. Econ. Ent. 35: 83-101.
- Mukerji, M.K. 1971. Major factors in survival of the immature stages of Hylemya brassicae (Diptera: Anthomyiidae) on cabbage. Can. Ent. 103: 717-728.
- Neilson, C.L. and J.C. Arrand. 1959. Field crop and vegetable insects: cabbage maggot. In: Handbook of the Main Economic Insects of B.C. pp: 12-13.
- Pimentel, D. 1961. An ecological approach to the insecticide problem. J. Econ. Ent. 54: 108-114.
- Pond, D.D., L.A. Dionne, R.G. White, C.A. Moore. 1962. Notes on egg-laying response of two species of root maggots on turnips bred for resistance to the cabbage root maggot, Hylemya brassicae (Bouché). Canadian Journal of Plant Science. 42: 530-531.

- Pont, A.C. 1981. The linnaean species of the families Fanniidae, Anthomyiidae and Muscidae (Insecta: Diptera). Biological Journal of the Linnean Society. 15: 165-175.
- Read, D.C. 1958. Notes on Scatophaga stercoraria (L.) (Diptera: Anthomyiidae), A Predator of the cabbage maggot, Hylemya brassicae (Bouché). Can. Ent. 90: 376.
- Read, D.C. 1960. Effects of soil treatment of heptachlor and parathion on predators and parasites of root maggots attacking rutabagas on Prince Edward Island. J. Econ. Ent. 53: 932-935.
- Read, D.C. 1962. Notes on the life history of Aleochara bilineata (Gyll.) (Coleoptera: Staphylinidae), and on its potential value as a control agent for the cabbage maggot, Hylemya brassicae (Bouché) (Diptera: Anthomyiidae). Can. Ent. 94: 417-425.
- Read, D.C. 1969. Rearing the cabbage maggot with and without diapause. Can. Ent. 101: 725-737.
- Read, D.C. and H.E. Welch. 1962. Establishing and maintaining a culture of Hylemya brassicae (Bouché) (Diptera: Anthomyiidae) in the greenhouse or laboratory. Can. Ent. 94: 458-460.
- Savage, H.J. 1989. Insect control: root maggots. In: Production Guide for Commercial Growers. Ministry of Agriculture and Fisheries, B.C., Canada. pp.31-32.
- Schoene, W.J. 1916. The cabbage maggot, its biology and control. Bull. N.Y. St. Agric. Exp. Stn. No.419: 104-124.
- Smith, K.M. 1927a. An unusual form of parasitism of an anthomyiid fly. Parasitology, 19: 260-262.
- Smith, K.M. 1927b. A study of Hylemya (Chortophila) brassicae Bouché, the cabbage root fly and its parasites. With notes on some other dipterous pests of cruciferous plants. Ann. Appl. Biol. 14: 312-329.
- Swales G.E. 1959. Resistance in rutabagas to the cabbage maggot, Hylemya brassicae (Bouché) (Diptera: Anthomyiidae). Can. Ent. 91: 700-703.
- Swales, G.E. 1961. Laboratory studies on mating and oviposition of Hylemya brassicae (Bouché) (Diptera: Anthomyiidae). The Canadian Entomologist. 93: 940-943.
- Swales, G.E. 1966. Sterilization of the cabbage maggot with apholate. J. Econ. Ent. 59: 596-598.

- Traynier, R.M.M. 1967. Stimulation of oviposition by the cabbage root fly Erioischia brassicae. Ent. exp. & appl. 10: 401-412.
- Varis, A.L. 1967. Studies on the biology of the cabbage root fly (Hylemya brassicae Bouché) and the turnip root fly. Ann. Agric. Fenn. 6: 1-13.
- Way, M.J. and G. Murdie. 1965. An example of varietal variations in resistance of Brussels sprouts. Ann. Appl. Biol. 56: 326-328.
- Wishart, G., J.F. Doane, and G.E. Maybee. 1956. Notes on beetles as predators of eggs of Hylemya brassicae (Bouché) (Diptera: Anthomyiidae). Can. Ent. 88: 634-639.
- Wishart, G. 1957. Surveys of parasites of Hylemya spp. (Diptera, Anthomyiidae) that attack cruciferous crops in Canada. Can. Ent. 89: 450-454.
- Wright, D.W. 1954. Cabbage root fly on summer cauliflower. Grower. 41: 293-296.

2. On the Nematodes

- Akhurst, R.J. 1980. Morphological and functional dimorphism in Xenorhabdus spp., bacteria symbiotically associated with the insect pathogenic nematodes Neoaplectana and Heterorhabditis. J. Gen. Microbiol. 121: 303-309.
- Akhurst, R.J. 1982a. A Xenorhabdus sp. (Eubacteriales: Enterobacteriaceae) symbiotically associated with Steinernema kraussei (Nematoda: Steinernematidae). Revue Nematol. 5(2): 277-280.
- Akhurst, R.J. 1982b. Antibiotic activity of Xenorhabdus spp., bacteria symbiotically associated with insect pathogenic nematodes of the families Heterorhabditidae and Steinernematidae. J. Gen. Microbiol. 128: 3061-3065.
- Akhurst, R.J. 1983. Taxonomic study of Xenorhabdus, a genus of bacteria symbiotically associated with insect pathogenic nematodes. Int. J. Syst. Bacteriol. 33: 38-45.
- Akhurst, R.J. 1986a. Xenorhabdus nematophilus subsp. beddingii (Enterobacteriaceae): a new subspecies of bacteria mutualistically associated with entomopathogenic nematodes. Int. J. Syst. Bacteriol. 36: 454-457.
- Akhurst, R.J. 1986b. Recent advances in Xenorhabdus research. In R.A. Samson, J.M. Vlak and D. Peters (eds). Fundamental and applied aspects of invertebrate pathology. Proc. IVth Int. Colloq. Invertebr. Pathol., Veldhoven, Netherlands, pp. 304-307.

- Akhurst, R.J. 1986c. Xenorhabdus nematophilus subsp. poinarii: its interaction with insect pathogenic nematodes. Syst. Appl. Microbiol. 8: 142-147.
- Akhurst, R.J. 1986d. Controlling insects in soil with entomogenous nematodes. In: R.A. Samson, J.V. Vlak and D. Peters (Editors), Fundamental and applied aspects of invertebrate pathology. Proc. IVth Int. Colloq. Invertebr. Pathol., Veldhoven, Netherlands, pp. 265-267.
- Baldwin, J.G. and H. Hirschmann. 1975. Fine structure of cephalic sense organs in Heterodera glycines males. J. Nematol. 7: 40-53.
- Bedding, R.A. 1976. New methods increase the feasibility of using Neoaplectana spp. (Nematoda) for the control of insect pests. Proc. First Int. Coll. Invertebr. Pathol., Kingston, Canada, pp.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. Ann. Appl. Biol. 104: 117-120.
- Bedding, R.A. and L.A. Miller. 1981. Use of a nematode, Heterorhabditis heliothidis, to control black vine weevil, Otiorhynchus sulcatus, in potted plants. Ann. Appl. Biol. 99: 211-216.
- Bedding, R.A. and A.S. Molyneux. 1982. Penetration of insect cuticle by infective juveniles of Heterorhabditis spp. (Heterorhabditidae: Nematoda). Nematologica 28: 354-359.
- Bedding, R.A., A.S. Molyneux and R.J. Akhurst. 1983. Heterorhabditis spp., Neoaplectana spp., and Steinernema kraussei: interspecific and intraspecific differences in infectivity for insects. Exp. Parasitol. 55: 249-257.
- Borner, H. 1960. Liberation of organic substances from higher plants and their role in the soil sickness problem. The Botanical Review 26(3): 393-424.
- Burman, M. and A.E. Pye. 1980. Neoaplectana carpocapsae: respiration of infective juveniles. Nematologica 26: 214-219.
- Byers, J.A. and G.O. Poinar Jr. 1982. Location of insect hosts by the nematode, Neoaplectana carpocapsae, in response to temperature. Behaviour 79(1): 1-10.
- Curran, J. 1989. Chromosome numbers of Steinernema and Heterorhabditis species. Revue Nematol. 12(2): 145-148.

- Dunphy, G.B. and J.M. Webster. 1984. Interaction of Xenorhabdus nematophilus subsp. nematophilus with the haemolymph of Galleria mellonella. J. Insect Physiol. 30(11): 883-889.
- Dunphy, G.B. and J.M. Webster. 1986. Influence of the Mexican strain of Steinernema feltiae and its associated bacterium Xenorhabdus nematophilus on Galleria mellonella. J. Parasit. 72(1): 130-135.
- Dunphy, G.B. and J.M. Webster. 1987. Partially characterized components of the epicuticle of dauer juvenile Steinernema feltiae and their influence on hemocyte activity in Galleria mellonella. J. Parasitol. 73(3): 584-588.
- Dunphy, G.B. and J.M. Webster. 1989. The monoxenic culture of Neoplectana carpocapsae DD 136 and Heterorhabditis heliothidis. Revue Nematol. 12(2): 113-123.
- Dutky, S.R., J.V. Thompson and G.E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol. 6: 417-422.
- Epsky, N.D., D.E. Walter and J.L. Capinera. 1988. Potential role of nematophagous microarthropods as biotic mortality factors of entomogenous nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). J. Econ. Entomol. 81(3): 821-825.
- Gaugler, R. 1981. Biological control potential of neoaplectanid nematodes. J. Nematol. 13: 241-249.
- Gaugler, R. 1988. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agriculture, Ecosystems and Environment 24: 351-360.
- Gaugler, R. and G.M. Boush. 1978. Effects of ultraviolet radiation and sunlight on the entomogenous nematode, Neoplectana carpocapsae. J. Invertebr. Pathol. 32: 291-296.
- Gaugler, R. and G.M. Boush. 1979. Laboratory tests on ultraviolet and protectants of an entomogenous nematode. Environ. Entomol. 8: 810-813.
- Gaugler, R., L. LeBeck, B. Nakagaki and G.M. Boush. 1980. Orientation of the entomogenous nematode Neoplectana carpocapsae to carbon dioxide. Environ. Entomol. 9: 649-652.
- Gaugler, R., T. McGuire, and J. Campbell. 1989. Genetic variability among strains of the entomopathogenic nematode Steinernema feltiae. J. Nematol. 21(2): 247-253.
- Georgis, R. and G.O. Poinar, Jr. 1983a. Effect of soil texture on the distribution and infectivity of Neoplectana

- 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(4): 652-654.
- Georgis, R. and N.G.M. Hague. 1981. A neoaplectanid nematode in the larch sawfly, Cephalcia lariciphila (Hymenoptera: Pamphiliidae). Ann. App. Biol. 99: 171-177.
- Hara, A.H. and H.K. Kaya. 1983. 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 dog food/agar medium. U.S. Dept. Agric., AAT-W-16.
- Hotchkin, P.G. and H.K. Kaya. 1984. Electrophoresis of soluble proteins from two species of Xenorhabdus, bacteria mutualistically associated with the nematodes Steinernema spp. and Heterorhabditis spp. J. Gen. Microbiol. 130: 2725-2731.
- Kaya, H.K. 1977. Development of the DD-136 strain of Neoaplectana carpocapsae at constant temperatures. J. Nematol. 9: 346-349.
- Kaya, H.K. 1985. Entomogenous nematodes for insect control in IPM systems. In M.A. Hoy and D.C. Herzog (eds). Biological control in agricultural IPM systems. New York: Academic Press, Inc.
- Kaya, H.K., J.M. Marston, J.E. Lindegren and Y.S. Peng. 1982. Low susceptibility of the honey bee, Apis mellifera L. (Hymenoptera: Apidae), to the entomogenous nematode, Neoaplectana carpocapsae Weiser. Environ. Entomol. 11: 920-924.
- Kaya, H.K. and R.C. Reardon. 1982. Evaluation of Neoaplectana carpocapsae for biological control of the western spruce budworm, Choristoneura occidentalis: ineffectiveness and persistence of tank mixes. J. Nematol. 14: 595-597.
- Khan, A., W.M. Brooks and H. Hirschmann. 1976. Chromonema heliothidis n. gen., n. sp. (Steinernematidae: Nematoda), a parasite of Heliothis zea (Noctuidae, Lepidoptera), and other insects. J. Nematol. 8(2): 159-168.

- Kondo, E. and N. Ishibashi. 1985. Effects of soil moisture on the survival and infectivity of the entomogenous nematode Steinernema feltiae (DD-136). Proc. Assoc. Pl. Prot. Kyushu 31: 186-190.
- Kondo, E. and N. Ishibashi. 1986. Nictating behavior and infectivity of entomogenous nematodes, Steinernema spp., to the larvae of common cutworm, Spodoptera litura (Lepidoptera: Noctuidae), on the soil surface. Appl. Ent. Zool. 21: 553-560.
- MacVean, C.M., J.W. Brewer and J.L. Capinera. 1982. Field tests of antidesiccants to extend the infection period of an entomogenous nematode, Neoaplectana carpocapsae, against the Colorado potato beetle. J. Econ. Entomol. 75: 97-101.
- Mankau, R. 1980. Biological control of nematode pests by natural enemies. Ann. Rev. Phytopathol. 18: 415-440.
- Milstead, J.E. and G.O. Poinar, Jr. 1978. A new entomogenous nematode for pest management systems. Calif. Agric. 32(3):12.
- Milstead, J.E. 1979. Heterorhabditis bacteriophora as a vector for introducing its associated bacterium into the hemocoel of Galleria mellonella larvae. J. Invertebr. Pathol. 33: 324-327.
- Molyneux, A.S. 1984. The influence of temperature on the infectivity of heterorhabditid and steinernematid nematodes for larvae of the sheep blowfly, Lucilia cuprina. Proc. 4th Aust. Appl. Entomol. Res. Conf., Adelaide, pp. 344-351.
- Molyneux, A.S. 1985. Survival of infective juveniles of Heterorhabditis spp., and Steinernema spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. Rev. Nematol. 8: 165-170.
- Molyneux, A.S. 1986. Heterorhabditis spp. and Steinernema (= Neoaplectana) spp.: temperature, and aspects of behavior and infectivity. Exp. Parasitol. 62: 169-180.
- Molyneux, A.S. and R.A. Bedding. 1985. 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.
- Molyneux, A.S., R.A. Bedding and R.J. Akhurst. 1983. Susceptibility of larvae of the sheep blowfly Lucilia cuprina to various Heterorhabditis spp., Neoaplectana spp., and an undescribed steinernematid (Nematoda). J. Invertebr. Pathol. 42: 1-7.
- Moyle, P.L. and H.K. Kaya. 1981. Dipersal and infectivity of the entomogenous nematode, Neoaplectana carpocapsae Weiser

(Rhabditida: Steinernematidae), in sand. J. Nematol. 13: 295-300.

Mracek, Z., R. Hanzal and D. Kodril. 1988. Sites of penetration of juvenile steinernematids and heterorhabditids (Nematoda) into the larvae of *Galleria mellonella* (Lepidoptera). J. Invertebr. Pathol. 52: 477-478.

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. 1967. Description and taxonomic position of the DD-136 nematode (Steinernematidae, Rhabditoidea) and its relationship to *Neoaplectana carpocapsae* Weiser. Proc. Helminthol. Soc. 34(2): 199-209.

Poinar, G.O.Jr. 1975. Entomogenous nematodes. Leiden, Netherlands: E.J. Brill.

Poinar, G.O.Jr. 1979. Nematodes for biological control of insects. Boca Raton, Florida: CRC Press, Inc.

Poinar, G.O., Jr., G.M. Thomas, S.B. Presser and J.L. Hardy. 1982. Inoculation of entomogenous nematodes, *Neoaplectana* and *Heterorhabditis* and their associated bacteria, *Xenorhabdus* spp. into chicks and mice. Environ. Entomol. 11: 137-138.

Poinar, G.O.Jr. and G.M. Thomas. 1965. A new bacterium, *Achromobacter nematophilus* sp. nov. (Achromobacteriaceae: Eubacteriales) associated with a nematode. Int. Bull. Bacteriol. Nomen. Taxon. 15: 249-252.

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). Parasitology. 56: 385-390.

Poinar, G.O.Jr., G.M. Thomas and R. Hess. 1977. Characteristics of the specific bacterium associated with *Heterorhabditis bacteriophora* (Heterorhabditidae: Rhabditida). Nematologica 23: 97-102.

Poinar, G.O.Jr. and H.B. Jansson. 1986a. Susceptibility of *Neoaplectana* spp. and *Heterorhabditis heliothidis* to the endoparasitic fungus, *Drechmeria coniospora*. J. Nematol. 18: 225-230.

Poinar, G.O.Jr. and H.B. Jansson. 1986b. Infection of *Neoaplectana* and *Heterorhabditis* (Rhabditida: Nematoda) with the predatory fungi, *Monacrosporium elliposporum* and *Arthrobotrys oligospora* (Moniliales: Deuteromycetes). Revue Nematol. 9(3): 241-244.

- Poinar, G.O.Jr and R. Leutenegger. 1968. Anatomy of the infective and normal third-stage juveniles of Neoaplectana carpocapsae Weiser (Steinernematidae: Nematoda). J. Parasitol. 54: 340-350.
- Pye, A.E. and M. Burman. 1978. Neoaplectana carpocapsae: infection and reproduction in large pine weevil larvae, Hylobius abietis. Exp. Parasitol. 46: 1-11.
- Pye, A.E. and M. Burman. 1981. Neoaplectana carpocapsae: nematode accumulations on chemical and bacterial gradients. Exp. Parasitol. 51: 13-20.
- Sandner, H. 1986. Movement of invasive larvae of Heterorhabditis bacteriophora Poinar in soil. Animal Science 20: 45-47.
- Schmeige, D.C. 1963. The feasibility of using a neoaplectanid nematode for control of some forest insect pests. J. Econ. Entomol. 56: 427-431.
- 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.
- Schroeder, W.J. and J.B. Beavers. 1987. Movement of the entomogenous nematodes of the families Heterorhabditidae and Steinernematidae in soil. J. Nematol. 19: 257-259.
- Shapiro, M., W. Mclane and R. Bell. 1985. Laboratory evaluation of selected chemicals as antidesiccants for the protection of the entomogenous nematode, Steinernema feltiae (Rhabditida: Steinernematidae), against Lymantria dispar (Lepidoptera: Lymantriidae). J. Econ. Entomol. 78: 1437-1441.
- Simons, W.R. and G.O. Poinar, Jr. 1973. The ability of Neoaplectana carpocapsae (Steinernematidae: Nematodea) to survive extended periods of desiccation. J. Invertebr. Pathol. 22: 228-230.
- Thomas, G.M. and G.O. Poinar, Jr. 1979. Xenorhabdus gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. International Journal of Systematic Bacteriology. 29(4): 352-260.
- Thurston, G.S. and W.N. Yule. 1990. Control of larval northern corn rootworm (Diabrotica barberi) with two steinernematid nematode species. J. Nematol. 22(1): 127-131.
- Timper, P., H.K. Kaya, and R. Gaugler. 1988. Dispersal of the entomogenous nematode Steinernema feltiae (Rhabditida:

Steinernematidae) by infected adult insects. Environ. Entomol. 17(3): 546-550.

Ward, S.. 1973. Chemotaxis by the nematode Caenorhabditis elegans: indentification of attractants and analysis of the response by use of mutants. Proc. Nat. Acad. Sci. USA. 70(3): 817-821.

Webster, J.M. and J.F. Bronskill. 1968. Use of Gelgard M and an evaporation retardant to facilitate control of larch sawfly by a nematode-bacterium complex. J. Econ. Entomol. 61(5): 1370-1373.

Welch, H.E. and L.J. Briand. 1961a. Tests of the nematode DD 136 and an associated bacterium for control of the Colorado potato beetle, Leptinotarsa decemlineata (Say). Can. Ent. 93: 759-763.

Welch, H.E. and L.J. Briand. 1961b. Field experiment on the use of a nematode for the control of vegetable crop insects. Proc. Entomol. Soc. Ont. 91: 197-202.

Woodring, J.L. and H.K. Kaya. 1988. Steinernematid and heterorhabditid nematodes. Southern Cooperative Series Bulletin 331, 30 pp. Arkansas Agricultural Experiment Station, Fayetteville, Arkansas, U.S.A.

Wouts, W.M. 1980. Biology, life cycle and redescription of Neoplectana bibionis Bovien, 1937 (Nematoda: Steinernematidae). J. Nematol. 12(1): 62-72.

Wouts, W.M. 1984. Nematode parasites of lepidopterans. In: Nickle, W.R. (ed) Plant and Insect Nematodes. Marcel Dekker Inc., New York and Basel. pp. 655-696.

Wouts, W.M., Z. Mracek, S. Gerdin and R.A. Bedding. 1982. Neoplectana Steiner, 1929 a junior synonym of Steinernema Travassos, 1927 (Nematoda; Rhabditida). Syst. Parasitol. 4: 147-154.

Zervos, S. and J.M. Webster. 1989. Susceptibility of the cockroach Periplaneta americana to Heterorhabditis heliothidis (Nematoda: Rhabditoidea) in the laboratory. Can. J. Zool. 67: 1609-1611.

3. On Statistics

Goldstein, A. 1964. Biostatistics: an introductory text. The Macmillan Company, New York; Collier-Macmillan Ltd., London. pp. 93-101.

APPENDIXES

Appendix 1. Infectivity of four nematode strains, namely DD136 and Breton of Steinernema, and NC162 and A13-5 of Heterorhabditis for first- and second-instar larval Delia radicum. There were five replicates (n=5) for each strain.

Treatments	DD136		Breton		NC162		A13-5		Control	
By day	5	6	5	6	5	6	5	6	5	6
Cumulative mortality(%)	28	72	36	90	72	80	56	88	22	58
% maggots infected with nematodes	18	46	16	22	46	52	38	64	0	0

Appendix 2. The number of nematodes, at three per dish, of Heterorhabditis heliothidis strain T327 which were present at newly-formed puparia of Delia radicum over time, at 21 C.

Dish	Time after nematode introduction									
	5 m	15 m	30 m	1 h	1.5h	2 h	2.5 h	3 h	3.5 h	4 h
A	0	0	0	0	1	1	1	1	1	1
B	0	0	0	0	0	1	0	1	0	1
C	0	1	0	0	2	2	2	2	2	2
D	0	0	0	1	1	1	1	1	1	1
E	0	0	0	1	1	1	1	0	1	1
F	0	0	0	0	0	0	1	0	1	1
G	0	0	0	1	1	1	1	0	0	0
H	0	0	0	0	1	1	1	2	3	3
I	0	0	0	0	0	0	0	0	0	0
J	0	0	0	0	0	0	1	0	1	1
K	0	0	0	0	0	0	0	0	0	1
L	0	0	1	1	1	0	0	0	0	0
M	0	0	2	1	0	1	1	1	1	1
To- tals	0	1	3	5	8	9	10	8	11	13

Appendix 3. The number of nematodes, at three per dish, of Heterorhabditis heliothidis strain T327 which were present at frozen, dead, 3rd-instar larvae of Delia radicum over time, at 21 C.

Dish	Time after nematode introduction					
	5 m	15 m	30 m	1 h	2 h	4 h
A	0	0	0	0	0	1
B	0	1	1	3	3	3
C	0	1	2	1	1	3
D	0	0	0	2	2	2
E	0	0	0	0	2	2
F	0	0	0	0	1	1
G	0	0	0	0	1	2
H	0	0	0	0	1	1
I	0	0	1	0	0	0
J	0	0	1	2	2	2
K	0	0	2	3	3	3
L	0	0	1	1	3	3
Totals	0	2	8	12	19	23

Appendix 4. Percentages of introduced, two-month-old, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of newly-formed puparia of Delia radicum, and targets of the control, within 4 h, at 24-24.5 C

Dish	Numbers of nematodes introduced	Numbers of puparia of <u>Delia radicum</u>			Control
		one	two	four	
A	213	3.29	1.41	10.80	1.88
		0.94	0.94	16.43	0.47
B	340	2.35	1.76	4.71	1.76
		9.41	3.82	4.41	0
C	362	0.83	2.76	4.14	2.21
		2.21	2.76	1.66	1.10
D	314	1.59	4.14	1.59	0.64
		1.27	6.05	2.87	0.32
E	373	6.43	3.72	8.58	0.80
		4.56	4.29	8.31	0
Mean		3.29	3.17	6.35	0.92
SE		0.88	0.49	1.48	0.25

Appendix 5. Percentages of introduced, newly-harvested, infective juvenile Heterorhabditis strain NC162, which reached targets of newly-formed puparia of Delia radicum, and targets of the control, within 24 h, at 23.5-25.8 C.

Dish	Numbers of nematodes introduced	Numbers of puparia of <u>Delia radicum</u>			Control
		one	two	four	
A	245	1.22	0.41	2.86	0
		0	1.22	10.61	0
B	244	0	2.87	3.69	0
		0.82	0.82	7.38	0
C	229	0	0.87	1.75	0
		1.31	1.75	7.86	0.44
D	266	0.38	1.13	2.63	0
		0.75	6.02	7.14	0
E	249	2.41	4.42	6.83	0
		0.40	0.80	2.81	0
Mean		0.73	2.03	5.36	0.04
± SE (%)		±0.24	±0.59	±0.94	±0.04

Appendix 6. Percentages of introduced, one-month-old, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of newly-formed puparia of Delia radicum, and targets of the control, within (a) 4 h at 23.5-25 C, and (b) 24 h at 23.3-25.1 C.

(a):

Dish	Numbers of nematodes introduced	Numbers of puparia of <u>Delia radicum</u>			Control
		one	two	four	
A	260	3.85	1.15	0.77	0.39
		0.39	0.77	2.31	1.15
B	278	0.72	1.08	1.80	0.72
		1.08	0	3.24	0
C	288	2.08	1.04	1.39	0
		0.35	1.04	2.08	1.04
D	281	1.07	2.14	2.14	0.36
		0.71	0.71	1.78	0
E	285	0.70	0.70	3.51	1.40
		1.40	2.46	0.70	0.35
Mean		1.24	1.11	1.97	0.54
± SE (%)		±0.33	±0.23	±0.29	±0.16

Appendix 6. Legend as on page 121 but

(b):

Dish	Numbers of nematodes introduced	Numbers of puparia of <u>Delia radicum</u>			Control
		one	two	four	
A	225	0	0.89	4.89	0
		0.89	3.11	2.67	0
B	252	0	1.19	2.78	0.40
		0.40	1.19	1.19	0
C	298	0	1.01	1.01	0
		0.34	0.34	2.01	0
D	318	0.31	0.31	0.31	0
		0	0.31	0.63	0
E	271	0.74	0.37	5.54	0
		0	0	2.21	0
Mean		0.27	0.87	2.32	0.04
± SE (%)		±0.11	±0.28	±0.55	±0.04

Appendix 7. Percentages of introduced, infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of larval Delia radicum and Galleria mellonella, and targets of the control, within 24 h, at 20-23.5 C.

Numbers of Target species and numbers of larvae						
Dish	nematodes introduced	<u>D. radicum</u> one	<u>D. radicum</u> four	<u>G. mellonella</u> one	<u>G. mellonella</u> four	Control
A	170	0	0.59	3.53	0	0
B	240	1.25	2.08	0	2.92	0
C	318	0	2.83	0.31	4.40	0
D	292	0.68	4.11	0	3.08	0
E	328	0	2.44	0.61	2.13	0
F	245	1.63	3.27	0	6.94	0
G	270	0	4.07	0.74	4.44	0
H	222	1.35	3.15	0.45	3.60	0
I	270	0	8.52	1.11	0.37	0
J	297	4.38	4.71	0	1.68	0
Mean		0.93	3.58	0.68	2.96	0
± SE (%)		±0.44	±0.66	±0.34	±0.65	0

Appendix 8. Numbers of dead maggots and numbers of maggots parasitized (in parentheses) by nematodes after exposing 1st-instar larval Delia radicum (at 10 per dish) to infective juvenile Heterorhabditis strain NC162, for 24 h and 48 h, at 20-22 C.

Dish	Treatment		Control	
	24 h	48 h	24 h	48 h
A	2 (1)	9 (8)	0	3
B	2 (2)	7 (7)	0	5
C	3 (3)	10 (10)	0	5
D	1 (1)	6 (4)	0	3
E	2 (2)	8 (7)	2	2
F	0 (0)	6 (6)	0	1
G	2 (2)	7 (5)	0	1
H	3 (3)	9 (9)	0	2
I	2 (2)	8 (7)	0	3
J	3 (2)	10 (8)	0	2
Mean	2 (1.8)	8 (7.1)	0.2	2.7
SE	0.30 (0.29)	0.47 (0.57)	0.2	0.45

Appendix 9*. The fate of 3rd-instar larval Delia radicum exposed to infective juvenile Heterorhabditis strain NC162 or T327, for 7 days, at 20-24.8 C.

Day	Group 1 (NC162)				Group 2 (T327)				Group 3 (control)			
	A	a	B	b	A	a	B	b	A	a	B	b
1	3	3	0	0	4	4	0	0	1	0	0	0
2	9	9	15	15	5	5	16	15	1	0	27	0
3	11	11	31	21	5	5	27	19	2	0	40	0
4	32	32	31	21	13	12	34	20	5	0	46	0
5	49	49	31	21	25	20	35	20	20	0	46	0
6	60	60	31	21	38	24	35	20	34	0	46	0
7	65	65	31	21	50	26	35	20	44	0	46	0

* Column A: number of dead larvae; Column a: number of larvae with nematodes; Column B: number of puparia; Column b: number of puparia with nematodes. All numbers are cumulative.

Appendix 10. Cumulative numbers of dead Delia radicum, pretreated with CO₂ (CO₂) and normal (N), then exposed to infective juvenile Heterorhabditis strain NC162 (at 500 or 5,000 per dish), and in the control (no nematodes), for 4 days, at 21.3-22.3 C.

L= 3rd-instar larvae; P= subsequently-formed puparia.

Treat- ments	By day	Petri dishes										Totals	Mean±SE (%)
		A		B		C		D		E			
		L	P	L	P	L	P	L	P	L	P		
5,000 CO ₂	1	10	0	10	0	9	0	9	0	9	0	47	94±2.45
	2	10	0	10	0	10	0	10	0	9	1	50	100±0
	3	10	0	10	0	10	0	10	0	9	1	50	100±0
	4	10	0	10	0	10	0	10	0	9	1	50	100±0
500 CO ₂	1	9	0	5	0	6	0	4	0	4	0	28	56±9.28
	2	10	0	6	0	6	0	7	0	6	0	35	70±7.75
	3	10	0	6	2	7	0	9	0	6	2	42	84±5.10
	4	10	0	6	2	7	0	9	0	6	2	42	84±5.10
Control CO ₂	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	1	1	0	2	4±2.45
	4	0	0	0	0	0	0	0	1	1	0	2	4±2.45
5,000 N	1	2	0	2	0	1	0	1	0	1	0	7	14±2.45
	2	5	3	6	1	2	3	2	3	3	5	33	66±6.78
	3	6	4	8	1	2	3	6	3	3	5	41	82±8.60
	4	6	4	8	1	2	3	6	3	4	5	42	84±8.72
500 N	1	1	0	2	0	0	0	2	0	0	0	5	10±4.47
	2	3	0	5	1	0	0	3	2	1	1	16	32±10.67
	3	4	1	5	1	2	1	4	2	2	2	24	48±5.83
	4	5	1	5	1	2	1	4	2	2	2	25	50±6.32
Control N	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	1	0	0	0	0	0	0	0	0	0	1	2±2.00
	4	1	0	0	0	0	0	0	0	0	0	1	2±2.00

Appendix 11a. The fate of adult Delia radicum exposed to infective juvenile Heterorhabditis strain NC162, and in the control, for 6 days, at 19-27 C.

Date (1988)		July 30 ^b	Aug.1	Aug.2	Aug.3	Aug.4
No. adults emerged	T ^c	44	83	85	88	88
	C ^c	40	82	86	88	88
% adults emerged	T	29.33	55.33	56.67	58.67	58.67
	C	26.67	54.67	57.33	58.67	58.
No. dead adults	T	26	54	63	78	86
	C	6	24	36	64	77
% adult mortality (no. dead/emerged)	T	59.09	65.06	74.12	88.64	97.73
	C	15.00	29.27	41.86	72.73	87.50
No. adults with nematodes	T	26	51	53	54	57
	C	0	0	0	0	0
% adults infected with nematodes	T	59.09	61.45	62.35	61.36	64.77
	C	0	0	0	0	0

a. Data are cumulative.

b. No examination on July 31.

c. T= treated with nematodes; C= control (no nematodes).

Appendix 12. Percentages of infective juvenile Heterorhabditis heliothidis strain T327, which reached targets of germinated seeds of radish (cv Cherry Belle), and of the control, within 3 h, at 24-25 C.

Dish	No. nematodes introduced	1 seed/ paper disc	3 seeds/ paper disc	Controls
A	135	0.74	2.22	0
		0.74	2.96	0.74
				0
B	157	1.91	2.55	0.64
		2.55	3.82	0
				0.64
C	159	2.52	5.03	0.63
		0	1.26	1.26
				0.63
D	162	4.32	3.09	1.26
		0.62	3.70	0
				0.62
E	179	2.79	2.23	0
		0.56	5.03	1.12
				0.56
Mean		1.68	3.19	0.46
± SE		± 0.43	± 0.39	± 0.15

Appendix 13. Percentages of infective juvenile Heterorhabditis heliothidis strain T327, which within 2.5 h reached: (a) root cylinders of radish and rutabaga, and the control, at 23.8-24.2 C; (b) root cylinders of radish and ball cabbage, and the control, at 23-24.4 C.

(a):

Dish	No. nematodes introduced	Radish	Rutabaga	Controls
A	297	13.80	1.68	0
		14.48	0	0.67 0.67 0
B	374	11.50	0.53	0.27
		16.31	1.60	0.27 0 1.07
C	433	10.39	0.46	0.69
		15.24	0.46	0 1.15 0.92
D	383	17.75	1.04	0.26
		20.37	1.04	0.26 0.52 0
E	424	9.67	0.94	1.18
		8.73	0.24	0.94 0.47 0.47
Mean		13.82	0.80	0.49
\pm SE		\pm 1.19	\pm 0.18	\pm 0.13

Appendix 13. Legend as on page 129 but

(b):

Dish	No. Nematodes introduced	Radish	Ball Cabbage	Controls
A	569	8.79	4.04	0.35
		6.85	9.14	0.53 0.18 0
B	482	7.47	12.86	0.41
		10.37	6.43	0.21 0.21 0.62
C	510	12.16	8.24	0.78
		8.63	6.27	0.59 0 0
D	415	6.02	7.71	0
		12.29	5.54	0.48 0.48 0.48
E	485	12.99	5.98	0
		8.45	5.36	0.21 0.82 0.21
Mean		9.40	7.16	0.33
± SE		± 0.77	± 0.79	± 0.09

Appendix 14. The results from the field trial treated with infective juvenile Heterorhabditis strain NC162: (a) number of larvae and puparia of Delia radicum recovered from the soil and radishes; (b) wet weight and number of radishes damaged by Delia radicum. (Explanation of the "Damage grades" is given in the text).
(a):

	Plot I		Plot II	
	Treatment I	Control I	Treatment II	Control II
Larvae	64	85	48	82
Puparia	0	22	10	27
Totals	64	107	58	109
Parasitized by nematodes	8	10	8	11

(b):

	Damage grades						Totals	Wet weight (g)	Mean wet weight (g)
	0	1	2	3	4	5			
Treatment I	5	12	25	28	19	5	94	3116.15	33.15
Control I	0	8	27	26	16	15	92	2899.13	31.51
Treatment II	12	24	28	15	9	8	96	3805.23	39.64
Control II	1	9	18	17	30	6	81	3700.68	45.69

Appendix 15. The results from the field trial treated with infective juvenile Heterorhabditis strain NC162: (a) number of larvae and puparia of Delia radicum recovered from the soil and roots of kohlrabi; (b) wet weight and number of kohlrabi damaged by Delia radicum. (Explanation of the "Damage grades" is given in the text).

(a):

	Plot I		Plot II	
	Treatment I	Control I	Treatment II	Control II
Larvae	90	82	111	91
Puparia	0	18	21	37
Totals	90	100	132	128
Parasitized by nematodes	1	2	0	0

(b):

	Damage grades						Totals	Wet Weight (g)	Mean wet weight (g)
	0	1	2	3	4	5			
Treatment I	1	10	19	23	20	6	79	2212.45	28.00
Control I	0	8	13	14	15	13	63	1942.37	30.83
Treatment II	2	5	11	30	25	3	76	3189.59	41.97
Control II	1	3	9	20	30	8	71	2458.70	34.63

Appendix 16. Correspondence from Dr. J. Curran (CSIRO).

Division of Entomology
Black Mountain, Canberra, ACT
Postal Address: GPO Box 1700, Canberra, ACT 2601
Telephone: (062) 46 4911. Telex: AA 62307. Fax: (062) 47 0217.
TELEEX AA62309
FAX 062-470217
INTERNATIONAL FAX 61-62-470217

TO: Zucheng Lei, Dept. of Biological Sciences

FROM: John Curran

COPIES TO:

DATE: 14/3/90

PAGE 1 OF 1 PAGES

SUBJECT: Taxonomy

Zucheng Lei
Department of Biological Sciences
Simon Fraser University

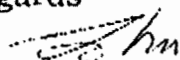
Dear Zucheng Lei

The taxonomy of *Heterorhabditis* is currently being revised. It has been proposed by George Poinar that *Heterorhabditis hellothidis* be synonymized with *Heterorhabditis bacteriophora*; furthermore it is proposed that *Heterorhabditis bacteriophora* redescribed by Wouts should be renamed *Heterorhabditis zealandica*. These, and other revisions, are to be published in the forthcoming CRC Press book, "Entomopathogenic nematodes in Biological Control" - ask Sandra for details on publication date, sometime in June. However, as your defence is before the publication date you would be obliged to use the current nomenclature i.e. *Heterorhabditis hellothidis*.

¹ As to NC162, it is my belief that it is a new species distinct from *Heterorhabditis hellothidis*, as is T327 which would fall within the proposed *Heterorhabditis zealandica*. However, since these thoughts are not yet published in a formal taxonomic sense you should observe the ICZN conventions and in formal writings refer to them as strains of *Heterorhabditis hellothidis*. However if you wish to you are most welcome to refer to my opinions as "pers. comms." ¹

I hope this is of use to you. Perhaps after George Poinars publication in June the taxonomy will be less of a mess.

Regards



John Curran

If you do not receive all of the pages shown above, please contact us as soon as possible by telephoning (062) 46 5587.